CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

International Chemical Identification: Extract from tea tree; Tea Tree Oil (TTO); Oil of *Melaleuca alternifolia* (Terpinen-4-ol Type)

EC Number: **285-377-1**

CAS Number: **68647-73-4**

Index Number: **Not allocated**

Contact details for dossier submitter: **Member state competent authority -PL**

Version number: 03 **Date: January 2022**

CONTENTS

1 IDENTITY OF THE SUBSTANCE

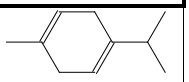
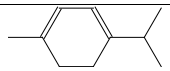
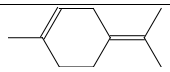
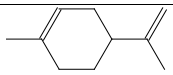
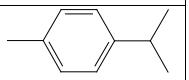
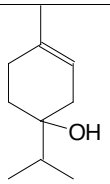
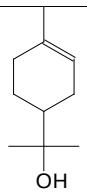
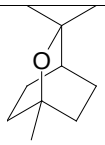
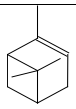
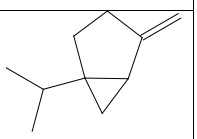
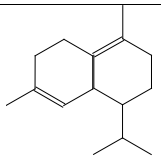
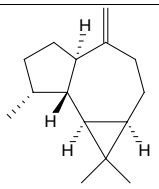
1.1 Name and other identifiers of the substance

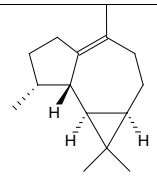
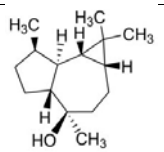
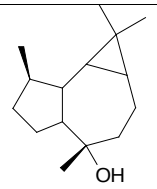
Table 1: Substance identity and information related to molecular and structural formula of the substance

<table>
<tr><td>Name(s) in the IUPAC nomenclature or other international chemical name(s)</td><td>Melaleuca alternifolia, ext. [1]
Melaleuca alternifolia, essential oil; tea tree oil [2]</td></tr>
<tr><td>Other names (usual name, trade name, abbreviation)</td><td>Tea Tree Oil, Oil of Melaleuca alternifolia (Terpinen-4-ol Type)
Extract from Tea tree,
Essential oil of melaleuca alternifolia</td></tr>
<tr><td>ISO common name (if available and appropriate)</td><td>-</td></tr>
<tr><td>EC number (if available and appropriate)</td><td>285-377-1 [1]
- [2]</td></tr>
<tr><td>CAS number (if available)</td><td>85085-48-9 [1]
68647-73-4 [2]
Tea Tree Oil (TTO) is naturally occurring substance having complex composition (UVCB). TTO is composed of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes and their associated alcohols.
For the components please refer to following table:
<table>
<tr><th>Name</th><th>CAS No.</th><th>EC No.</th></tr>
<tr><td>Terpinen-4-ol</td><td>562-74-3</td><td>209-235-5</td></tr>
<tr><td>γ-Terpinene</td><td>99-85-4</td><td>202-794-6</td></tr>
<tr><td>α-Terpinene</td><td>99-86-5</td><td>202-795-1</td></tr>
<tr><td>α-Terpineol</td><td>98-55-5</td><td>202-680-6</td></tr>
<tr><td>α-Terpinolene</td><td>586-62-9</td><td>209-578-0</td></tr>
<tr><td>α-Pinene</td><td>80-56-8</td><td>201-291-9</td></tr>
<tr><td>p-Cymene</td><td>99-87-6</td><td>202-796-7</td></tr>
<tr><td>1,8-Cineole (Eucalyptol)</td><td>470-82-6</td><td>207-431-5</td></tr>
<tr><td>Limonene</td><td>138-86-3</td><td>205-341-0</td></tr>
<tr><td>Aromadendrene</td><td>489-39-4</td><td>207-694-6</td></tr>
<tr><td>δ-Cadinene</td><td>483-76-1</td><td>--</td></tr>
<tr><td>Sabinene</td><td>3387-41-5</td><td>222-212-4</td></tr>
<tr><td>Globulol</td><td>489-41-8</td><td>207-696-7</td></tr>
<tr><td>Viridiflorol</td><td>552-02-3</td><td>209-003-3</td></tr>
<tr><td>Ledene</td><td>21747-46-6</td><td>244-565-3</td></tr>
</table></td></tr>
<tr><td>Other identity code (if available)</td><td>Not available.</td></tr>
<tr><td>Molecular formula</td><td>No molecular formula and mass can be assigned to Tea Tree Oil because it is a substance having complex composition. TTO is composed of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes and their associated alcohols. The specified components according to ISO 4730:2004 are displayed in the following table. Furthermore, the components contained in Tea Tree Oil can be grouped according to their chemical structure as several</td></tr>
</table>

	components have very similar structures. Consequently, their chemical properties within the assigned groups are comparable.

Structural formula

	Name	Molecular weight	Molecular formula	Structural formula
Monocyclic monoterpenes, Aliphatic and aromatic hydrocarbons	γ-Terpinene	136.24	$C_{10}H_{16}$	
	α-Terpinene	136.24	$C_{10}H_{16}$	
	α-Terpinolene	136.24	$C_{10}H_{16}$	
	Limonene	136.24	$C_{10}H_{16}$	
	p-Cymene	134.22	$C_{10}H_{14}$	
Monocyclic monoterpenes, aromatic unsaturated tertiary alcohols	Terpinen-4-ol	154.25	$C_{10}H_{18}O$	
	α-Terpineol	154.25	$C_{10}H_{18}O$	
Bicyclic monoterpenes	1,8-Cineole (Eucalyptol)	154.25	$C_{10}H_{18}O$	
	α-Pinene	136.24	$C_{10}H_{16}$	
	Sabinene	136.24	$C_{10}H_{16}$	
Polycyclic sesquiterpenes, Cadinane group	δ-Cadinene	204.35	$C_{15}H_{24}$	
Polycyclic sesquiterpenes Aromadendrene group	Aromadendrene	204.35	$C_{15}H_{24}$	

		Ledene (Viridiflorene)	204.35	$C_{15}H_{24}$	
	Polycyclic sesquiterpenes, Aromadendrene group Alcohols	Globulol	222.37	$C_{15}H_{26}O$	
		Viridiflorol	222.37	$C_{15}H_{26}O$	

SMILES notation (if available)	
Molecular weight or molecular weight range	No molecular formula and mass can be assigned to Tea Tree Oil because it is a substance having complex composition. TTO is composed of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes and their associated alcohols.
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable.
Description of the manufacturing process and identity of the source (for UVCB substances only)	TTO is manufactured by steam distillation of the leaves of the tea tree.

Degree of purity (%) (if relevant for the entry in Annex VI)

Name	Min. %	Max. %
Terpinen-4-ol	30	48
γ-Terpinene	10	28
α-Terpinene	5	13
α-Terpineol	1.5	8
α-Terpinolene	1.5	5
α-Pinene	1	6
p-Cymene	0.5	8
1,8-Cineole (Eucalyptol)	trace	15
Limonene	0.5	1.5
Aromadendrene	0.5	3
δ-Cadinene	trace	3
Sabinene	trace	3.5
Globulol	trace	1
Viridiflorol	trace	1
Ledene	trace	3

source: ISO 4730:2004[1]

[1] Oil of Melaleuca, Terpinen-4-ol type (Tea Tree Oil); International standard; ISO 4730:2004(E); Second edition 2004-10-01

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
Terpinen-4-ol	30 - 48	No entry in Annex VI	Flam. Liq. 3 H226 Acute Tox. 4 H302 Asp. Tox. 1 H304
γ-Terpinene	10 - 28	No entry in Annex VI	Flam. Liq. 3 H226 Asp. Tox. 1 H304
α-Terpinene	5 - 13	No entry in Annex VI	Flam. Liq. 3 H226 Acute Tox. 4 H302 Asp. Tox. 1 H304
α-Terpineol	1.5 - 8	No entry in Annex VI	Flam. Liq. 3 H226 Asp. Tox. 1 H304
α-Terpinolene	1.5 - 5	No entry in Annex VI	Flam. Liq. 3 H226 Asp. Tox. 1 H304
α-Pinene	1 - 6	No entry in Annex VI	Flam. Liq. 3 H226 Asp. Tox. 1 H304
p-Cymene	0.5 - 8	No entry in Annex VI	Flam. Liq. 3 H226 Asp. Tox. 1 H304
1,8-Cineole (Eucalyptol)	trace - 15	No entry in Annex VI	Flam. Liq. 3 H226 Acute Tox. 4 H302 Asp. Tox. 1 H304
Limonene	0.5 - 1.5	Flam. Liq. 3 H226 Skin Irrit. 2 H315 Skin Sens. 1 H317 Aquatic Acute 1 H400 Aquatic Chronic 1 H410	Flam. Liq. 3 H226 Skin Irrit. 2 H315 Aquatic Acute 1 H400 Aquatic Chronic 1 H410
Aromadendrene	0.5 - 3	No entry in Annex VI	**None**
δ-Cadinene	trace - 3	No entry in Annex VI	**None**
Sabinene	trace - 3.5	No entry in Annex VI	Flam. Liq. 3 H226 Asp. Tox. 1 H304
Globulol	trace - 1	No entry in Annex VI	**None**
Viridiflorol	trace - 1	No entry in Annex VI	**None**
Ledene	trace - 3	No entry in Annex VI	**None**

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
No relevant impurity exists for Tea Tree Oil.				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
None.					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5: Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	-	-	-	-	-	-	-	-	-	-	-
Dossier submitters proposal	TBD	Melaleuca alternifolia, ext. [1] *Melaleuca alternifolia*, essential oil; tea tree oil [2]	285-377-1 [1] - [2]	85085-48-9 [1] 68647-73-4 [2]	Flam. Liq. 3 Repr. 2 Acute Tox. 4 Acute Tox. 4 Asp. Tox. 1 Skin Irrit. 2 Skin Sens. 1B Aquatic Acute 1 Aquatic Chronic 3	H226 H361f H332 H302 H304 H315 H317 H400 H412	GHS02 GHS08 GHS07 GHS09 Dgr	H226 H361f H332 H302 H304 H315 H317 H410		oral: ATE = 1049 mg/kg bw inhalation: ATE= 3.64 mg/L (mist) M=1	-
Resulting Annex VI entry if agreed by RAC and COM	TBD	Melaleuca alternifolia, ext. [1] *Melaleuca alternifolia*, essential oil; tea tree oil [2]	285-377-1 [1] - [2]	85085-48-9 [1] 68647-73-4 [2]	Flam. Liq. 3 Repr. 2 Acute Tox. 4 Acute Tox. 4 Asp. Tox. 1	H226 H361f H332 H302	GHS02 GHS08 GHS07 GHS09 Dgr	H226 H361f H332 H302 H304		oral: ATE = 1049 mg/kg bw inhalation: ATE= 3.64 mg/L (mist)	

					Skin Irrit. 2 Skin Sens. 1B Aquatic Acute 1 Aquatic Chronic 3	H304 H315 H317 H400 H412		H315 H317 H410		M=1	

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	data conclusive but not sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	hazard class not applicable	No
Oxidising gases	hazard class not applicable	No
Gases under pressure	hazard class not applicable	No
Flammable liquids	harmonised classification proposed	Yes
Flammable solids	hazard class not applicable	No
Self-reactive substances	data conclusive but not sufficient for classification	Yes
Pyrophoric liquids	data conclusive but not sufficient for classification	Yes
Pyrophoric solids	hazard class not applicable	No
Self-heating substances	data conclusive but not sufficient for classification	Yes
Substances which in contact with water emit flammable gases	data conclusive but not sufficient for classification	Yes
Oxidising liquids	data conclusive but not sufficient for classification	Yes
Oxidising solids	hazard class not applicable	No
Organic peroxides	hazard class not applicable	No
Corrosive to metals	data conclusive but not sufficient for classification	Yes
Acute toxicity via oral route	harmonised classification proposed	Yes
Acute toxicity via dermal route	data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	harmonised classification proposed	Yes
Skin corrosion/irritation	harmonised classification proposed	Yes
Serious eye damage/eye irritation	data conclusive but not sufficient for classification	Yes
Respiratory sensitisation	hazard class not assessed in this dossier; data lacking	No
Skin sensitisation	data conclusive but not sufficient for classification	Yes
Germ cell mutagenicity	data conclusive but not sufficient for classification	Yes
Carcinogenicity	data conclusive but not sufficient for classification	Yes
Reproductive toxicity	data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	data conclusive but not sufficient for classification	Yes
Aspiration hazard	hazard class not assessed in this dossier; data lacking	Yes
Hazardous to the aquatic environment	harmonised classification proposed	Yes

Hazard class	Reason for no classification	Within the scope of public consultation
Hazardous to the ozone layer	hazard class not assessed in this dossier; data lacking	No

Tea Tree Oil is an active substance in the scope of the Regulation (EC) 1107/2009 (repealing Directive 91/414/EEC). The substance is not currently listed in Annex VI of CLP, and there have been no previous classification and labelling discussions of this substance. The substance is therefore subject to the harmonised classification and labelling process in accordance with Article 36(2) of CLP and no further justification is required.

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Tea Tree Oil (TTO) has not previously been assessed for harmonized classification by RAC or TC C&L.

TTO is not currently listed in Annex VI of Regulation (EC) 1272/2008.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level. TTO is an active substance in the meaning of Regulation EC 1107/2009 therefore is subject to harmonised classification and labelling according to Article 36 CLP Regulation.

5 IDENTIFIED USES

Tea Tree Oil is intended to be used large-scale in the field (e.g. tomato and grape) and in greenhouse (e.g. tomato) to control fungal diseases, e.g. powdery mildew and grey mold.

6 DATA SOURCES

Source of data are studies which have been submitted for Annex I renewal under 1107/2009 and studies that were evaluated under 91/414/EEC.

REACH registration dossier for melaleuca alternifolia, ext. (EC no 285-377-1), CSR, 2020.

Cosmetic Ingredient Review: https://cir-safety.org/sites/default/files/melalt092020SLR.pdf

BfR assessment of Tea tree oil used in cosmetics:

https://www.bfr.bund.de/cm/349/use_of_undiluted_tea_tree_oil_as_a_cosmetic.pdf

7 PHYSICOCHEMICAL PROPERTIES

For details of the summarized studies on physicochemical properties of Tea Tree Oil, please refer to Annex 1 of the CLH report.

Reliability statement: The data in the table below have been compiled from laboratory studies as well as open scientific literature. All laboratory studies have been evaluated for deviations from the respective test guidelines, accordance to quality criteria and overall scientific reliability. All the studies fulfilled their respective validity criteria. Only minor deviations have been identified in some of the studies which were not at all relevant for their scientific reliability and regulatory suitability. Hence, the laboratory studies are considered reliable (reliability score: 1).

The literature studies have not been performed according to official test guidelines. Therefore, it is not possible to evaluate their compliance by identifying any deviations or comparing them against guideline-specific quality criteria. Nonetheless, the publications in question have been assessed for regulatory relevance and scientific

reliability according to the criteria set out in the EFSA guidance for submission of scientific literature (EFSA Journal 2011;9(2):2092) and assigned a respective reliability score. Details of this process are described in the literature section (CA 9) of the active substance dossier, which clarifies that all studies used in the dossier were classified by the applicant as reliable (reliability score: 1) or reliable with restrictions (reliability score: 2, supporting information).

Table 7: Summary of physicochemical properties

<table>
<tr><th>Property</th><th>Value</th><th>Reference</th><th>Comment (e.g. measured or estimated)</th></tr>
<tr><td>Physical state at 20°C and 101.3 kPa</td><td>Colourless to pale yellow liquid</td><td>Anonymous 2016a</td><td>Observed</td></tr>
<tr><td rowspan="2">Melting/freezing point</td><td>No melting point down to - 100°C at atmospheric pressure</td><td>Anonymous 2016a</td><td>Measured</td></tr>
<tr><td>-22°C</td><td>ECHA dissemination site[2]</td><td>Measured (EU A.1)</td></tr>
<tr><td rowspan="2">Boiling point</td><td>177 – 191°C</td><td>Anonymous 2016a</td><td>Measured</td></tr>
<tr><td>97-220°C</td><td>ECHA dissemination site[3]</td><td>Measured (EU A.2)</td></tr>
<tr><td rowspan="2">Relative density</td><td>d_{20}^{20} = 0.902</td><td>Anonymous 2017</td><td>Measured</td></tr>
<tr><td>$D_{20/4}$ = 0.89</td><td>ECHA dissemination site[4]</td><td>Measured (EU A.3)</td></tr>
<tr><td>Vapour pressure</td><td>1,8-Cineole: 385 Pa at 20°C, 501 Pa at 25°C, 2048 Pa at 50°C
Terpinen-4-ol: 14.9 Pa at 20°C</td><td>Anonymous 2016b
Parsons, A.(2007)</td><td>Measured</td></tr>
<tr><td>
<table>
<tr><th>Components</th><th>Vapor pressure</th></tr>
<tr><td>γ-Terpinene</td><td>103 Pa at 23.5°C[1]; 145 Pa at 25°C*</td></tr>
<tr><td>α-Terpinene</td><td>145 Pa at 25°C*</td></tr>
<tr><td>α-Terpineol</td><td>5.69 Pa at 23.5°C[1]; 5.64 Pa at 25°C*</td></tr>
<tr><td>α-Terpinolene</td><td>99 Pa at 23.5°C[1]; 222 Pa at 25°C**</td></tr>
<tr><td>α-Pinene</td><td>544 Pa at 23.5°C[1]; 633 Pa at 25°C*</td></tr>
<tr><td>p-Cymene</td><td>219 Pa at 25°C*</td></tr>
<tr><td>Limonene</td><td>222 Pa at 23.5°C[1]; 192 Pa at 25°C*</td></tr>
<tr><td>Aromadendrene</td><td>5.27 Pa at 25°C**</td></tr>
<tr><td>δ-Cadinene</td><td>2.51 Pa at 25°C**</td></tr>
<tr><td>Sabinene</td><td>981 Pa at 25°C**</td></tr>
<tr><td>Globulol</td><td>0.00495 Pa at 25°C**</td></tr>
<tr><td>Viridiflorol</td><td>0.00495 Pa at 25°C**</td></tr>
<tr><td>Ledene</td><td>2.72 Pa at 25°C**</td></tr>
</table>
[1] Experimental value from a literature study
*From Episuite v4.11, experimental
** From Episuite v4.11, calculated
</td><td>Li, J., Perdure, E.M. et al., (1998)

Anonymous 2018</td><td>Measured, estimated</td></tr>
</table>

[2] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/3

[3] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/4

[4] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/5

<table>
<tr><th>Property</th><th>Value</th><th>Reference</th><th>Comment (e.g. measured or estimated)</th></tr>
<tr><td></td><td>The maximum vapour pressure of tea tree oil:
2100 Pa at 25°C
(vapour pressure readings of the samples with time show a decrease, thus the maximum reading from samples as the maximum vapour pressure of the test substance was reported)</td><td>ECHA dissemination site[5]</td><td>Measured (EU A.4)</td></tr>
<tr><td rowspan="2">Surface tension</td><td>45.6 mN/m for 1% w/v aqueous solution at 20°C
28.4 mN/m for neat Tee Tree Oil at 20 °C</td><td>Parsons, A. (2007)</td><td>Measured</td></tr>
<tr><td>51.0 mN/m of 90% saturated aqueous solution of tea tree oil at 20°C
55.0 mN/m of 1g/L aqueous solution of tea tree oil at 20°C</td><td>ECHA dissemination site[6]</td><td>Measured (EU A.5)</td></tr>
<tr><td rowspan="3">Water solubility</td><td>1,8-Cineole Solubility in water at 20°C:
2.76 g/L (pH = 5.2)</td><td>Anonymous 2016c</td><td>Measured</td></tr>
<tr><td>Terpinen-4-ol Solubility in water at 20°C:
3.28 g/L (pH = 5.85)</td><td>Parsons, A., (2007)</td><td>Measured</td></tr>
<tr><td>
<table>
<tr><th>Components</th><th>Solubility in water [mg/L]</th></tr>
<tr><td>α-Pinene</td><td>2.53 at 23.5 °C [1]</td></tr>
<tr><td>Limonene</td><td>6.32 at 23.5 °C [1]</td></tr>
<tr><td>γ-Terpinene</td><td>8.68 at 23.5 °C [1]</td></tr>
<tr><td>Terpinolene</td><td>9.48 at 23.5 °C [1]</td></tr>
<tr><td>α-Terpineol</td><td>626.7 at 23.5 °C [1]
1980 *</td></tr>
<tr><td>p-Cymene</td><td>23.4 at 25 °C [2]</td></tr>
<tr><td>α-Terpinene</td><td>8.68 *</td></tr>
<tr><td>1,8-Cineole</td><td>3500*</td></tr>
<tr><td>Aromadendrene</td><td>0.07057**</td></tr>
<tr><td>δ-Cadinene</td><td>0.04863**</td></tr>
<tr><td>Sabinene</td><td>2.494**</td></tr>
<tr><td>Globulol</td><td>11.98**</td></tr>
<tr><td>Viridiflorol</td><td>11.98**</td></tr>
<tr><td>Ledene</td><td>0.07057**</td></tr>
</table>
[1] Experimental value from the literature study Li, J. Perdure, E.M. et al., 1998
[2] Experimental value from the literature study Banerjee, S.; Yalkowsky, S.H. & Valvani, S.C., 1980
*From Episuite v4.11, experimental database match
** From Episuite v4.11, estimated at 25°C
</td><td>Li, J. Perdure, E.M. et al., (1998)
Banerjee, S.; Yalkowsky, S.H. & Valvani, S.C., (1980)
Anonymous 2018</td><td>Measured, estimated</td></tr>
</table>

[5] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/7

[6] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/11

<table>
<tr><th>Property</th><th>Value</th><th>Reference</th><th>Comment (e.g. measured or estimated)</th></tr>
<tr><td></td><td>tea tree oil: 1420mg/L
Alpha-terpinene: 1.7 mg/l
p-cymene: 3.0 mg/l
Cineole: 73 mg/l
Gamma-terpinene: 3.0 mg/l
Terpinen-4-ol: 1140 mg/l
Alpha-terpineol: 87 mg/l at 20°C, pH=6.7</td><td>ECHA dissemination site[7]</td><td>Measured (EU A.6)</td></tr>
<tr><td rowspan="3">Partition coefficient n-octanol/water</td><td>Terpinen-4-ol
Log P_{OW} = 2.643 at 23.5°C and pH of 5.85.</td><td>Parsons, A. (2007)</td><td>Measured</td></tr>
<tr><td>
<table>
<tr><th>Components</th><th>Log Pow</th></tr>
<tr><td>Terpinen-4-ol</td><td>2.80[3]</td></tr>
<tr><td>α-Pinene</td><td>4.83[1];4.48[3]</td></tr>
<tr><td>Limonene</td><td>4.57[1];4.38[3]</td></tr>
<tr><td>γ-Terpinene</td><td>4.50[1];4.36[3]</td></tr>
<tr><td>Terpinolene</td><td>4.47[1];4.24[3]</td></tr>
<tr><td>α-Terpineol</td><td>2.98[1];3.28[3]</td></tr>
<tr><td>p-Cymene</td><td>6.34[2];;;4.10*</td></tr>
<tr><td>α-Terpinene</td><td>4.25[3]</td></tr>
<tr><td>1,8-Cineole</td><td>2.74[3]</td></tr>
<tr><td>Aromadendrene</td><td>6.13**</td></tr>
<tr><td>δ-Cadinene</td><td>6.32**</td></tr>
<tr><td>Sabinene</td><td>4.69**</td></tr>
<tr><td>Globulol</td><td>4.63**</td></tr>
<tr><td>Viridiflorol</td><td>4.63**</td></tr>
<tr><td>Ledene</td><td>6.18**</td></tr>
</table>
[1] Experimental value from the literature study Li, J. Perdure, E.M. et al., 1998
[2] Experimental value from the literature study Banerjee, S.; Yalkowsky, S.H. & Valvani, S.C. 1980
[3] Experimental value from the literature study Griffin, S.; Wyllie, S.G. & Markham, J., 1999
*From Episuite v4.11, experimental database match
** From Episuite v4.11, estimated</td><td>Li, J. Perdure, E.M. et al., 1(998)

Banerjee, S.; Yalkowsky, S.H. & Valvani, S.C. (1980)

Griffin, S.; Wyllie, S.G. & Markham, J., (1999)</td><td>Measured, estimated</td></tr>
<tr><td>The range of partition coefficient values for Tea Tree Oil was found to be 2510 to 314000 (logPow= 3.4 to 5.5) at 30°C.
Alpha-terpineol: logPow = 3.4
Terpinen-4-ol: logPow = 3.5
Alpha-terpinene: logPow = 5.2
Gamma-terpinene: logPow = 5.3
The effect of pH on the partition coefficient was not assessed since the test substance does not possess a dissociation constant in environmental pH ranges.</td><td>ECHA dissemination site[8]</td><td>Measured (EU A.8)</td></tr>
</table>

[7] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/9

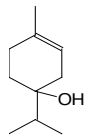
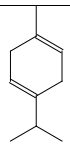
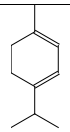
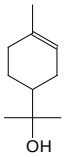
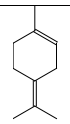
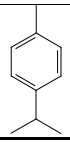
[8] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/8

Property	Value	Reference	Comment (e.g. measured or estimated)
Flash point	55°C at 1007 mbar	Parsons, A. (2007)	Measured
	54°C at 1022 mbar	ECHA dissemination site[9]	Measured (EU A.9) closed cup method
	55°C at 1021 mbar	ECHA dissemination site[10]	Measured (EU A.9) Equilibrium method
Flammability	Auto-ignition temperature is 269°C at 1008 mbar	Parsons, A. (2007)	Measured
Explosive properties	No likely or realistic possibility of this substance being an explosive hazard.	Parsons, A. (2007)	Reasoned case
Self-ignition temperature	Auto-ignition temperature is 269°C at 1008 mbar.	Parsons, A. (2007)	Measured
	The auto-ignition temperature of Tea Tree Oil: 252°C at 1020 mbar	ECHA dissemination site[11]	Measured (EU A.15) auto-ignition temperature-liquids
Oxidising properties	No likely or realistic possibility of this substance being an oxidation hazard.	Parsons, A. (2007)	Reasoned case
Granulometry	not relevant		
Stability in organic solvents and identity of relevant degradation products	Tea Tree Oil: Solubility at 20°C: n-Heptane: > 25 – 29 g/L Acetone: ≥ 500 g/L Dichloroethane: ≥ 500 g/L Ethyl acetate: ≥ 500 g/L Methanol: ≥ 500 g/L p-Xylene: 50 – 57 g/L	Anonymous2016d	Measured
	Terpinen-4-ol: Solubility at 20°C: n-Heptane: > 250 g/L Acetone: > 250 g/L Dichloroethane: > 250 g/L Ethyl acetate: > 250 g/L Methanol: > 250 g/L p-Xylene: > 250 g/L	Parsons, A. (2007)	Measured

[9] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/12

[10] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/12/?documentUUID=4e36aeff-be3a-44cc-a78d-934d00ed43c2

[11] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/13

<table>
<tr><th>Property</th><th>Value</th><th>Reference</th><th>Comment (e.g. measured or estimated)</th></tr>
<tr><td>Dissociation constant</td><td>
<table>
<tr><th>Name</th><th>Structural formula</th><th>Findings, Guideline, Method, Comments</th></tr>
<tr><td>Terpinen-4-ol</td><td>  </td><td>No dissociation constant available in data bases. No acidic hydrogen present in the molecule and dissociation not considered relevant at acid to slightly alkaline pH values.</td></tr>
<tr><td>γ-Terpinene</td><td>  </td><td>No dissociation is expected based on the molecular structure</td></tr>
<tr><td>α-Terpinene</td><td>  </td><td>No dissociation is expected based on the molecular structure</td></tr>
<tr><td>α-Terpineol</td><td>  </td><td>No dissociation constant available in data bases. No acidic hydrogen present in the molecule and dissociation not considered relevant at acid to slightly alkaline pH values.</td></tr>
<tr><td>α-Terpinolene</td><td>  </td><td>No dissociation is expected based on the molecular structure</td></tr>
<tr><td>α-Pinene</td><td>  </td><td>No dissociation is expected based on the molecular structure</td></tr>
<tr><td>p-Cymene</td><td>  </td><td>No dissociation is expected based on the molecular structure</td></tr>
</table>
</td><td>--</td><td>Statement based on the structure</td></tr>
<tr><td>Viscosity</td><td>Kinematic viscosity of tea tree oil:
2.86 mm2/s at 20°C
1.71 mm2/s at 40°C
Dynamic viscosity:
2.54 mPa/s at 20°C
1.52 mPa/s at 40°C</td><td>ECHA dissemination site[12]</td><td>Measured (OECD TG 114) using a reverse flow viscometer</td></tr>
</table>

[12] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/23

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 8: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
Reasoned case	No likely or realistic possibility of this substance being an Explosive Hazard	-	Parsons, A. (2007)

8.1.1 Short summary and overall relevance of the information provided on explosive properties

As all the ingredients are, as natural products, clearly substances of long standing which have for a long time been used on a large scale, with no recorded problems in respect of explosivity, it would suggest that it is extremely unlikely to pose any threat from this perspective.

Reference to the Safety Literature (e.g. Sax[13], Bretherick[14] etc.) shows no suggestions of any explosive hazards associated with any of the materials present in the oil and the chemical structures of the compounds concerned do not contain any of the groups more likely to lead to explosivity problems (i.e. groups such as Nitrate, Perchlorate, Pierate etc.).

A sample of the product was subjected to Differential Scanning Calorimetry on a Perkin Elmer, Pyris 6 DSC. The sample was examined over the range 30°C to 400°C, programmed at a rate of 10°C/min and two replicate runs were carried out.

It was found that there were no significant exothermic events that occurred during this test, which would indicate that it is very unlikely that a thermally induced explosive reaction is likely to occur with this material.

It is therefore no likely or realistic possibility of this material being an explosive hazard.

8.1.2 Comparison with the CLP criteria

The substance does not meet the CLP criteria for classification for this hazard class.

8.1.3 Conclusion on classification and labelling for explosive properties

The substance does not have explosive properties. Data is conclusive but not sufficient for classification.

8.2 Flammable gases (including chemically unstable gases)

Hazard class is not applicable (TTO is not a gas).

8.2.1 Short summary and overall relevance of the provided information on flammable gases (including chemically unstable gases)

Hazard class not applicable: The substance is a liquid.

[13] Sax's Dangerous Properties of Industrial Materials, John Wiley & Sons, 2004, ISBN: 9780471476627

[14] Bretherick's Handbook of Reactive Chemical Hazards, Academic Press, 2006, ISBN: 9780123725639

8.2.2 Comparison with the CLP criteria

Hazard class not applicable: The substance is a liquid.

8.2.3 Conclusion on classification and labelling for flammable gases

Hazard class not applicable: The substance is a liquid.

8.3 Oxidising gases

Hazard class is not applicable (TTO is not a gas).

8.3.1 Short summary and overall relevance of the provided information on oxidising gases

Hazard class not applicable: The substance is a liquid.

8.3.2 Comparison with the CLP criteria

Hazard class not applicable: The substance is a liquid.

8.3.3 Conclusion on classification and labelling for oxidising gases

Hazard class not applicable: The substance is a liquid.

8.4 Gases under pressure

Hazard class is not applicable (TTO is not a gas).

8.4.1 Short summary and overall relevance of the provided information on gases under pressure

Hazard class not applicable: The substance is a liquid.

8.4.2 Comparison with the CLP criteria

Hazard class not applicable: The substance is a liquid.

8.4.3 Conclusion on classification and labelling for gases under pressure

Hazard class not applicable: The substance is a liquid.

8.5 Flammable liquids

Table 9: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
EC A9- Flash point	55°C	-	Parsons, A. (2007)
EU A.9 – flash point Equilibrium method	55°C at 1021 mbar	-	ECHA dissemination site[15]
EU A.9 – flash point	54°C at 1022 mbar	-	ECHA

[15] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/12/?documentUUID=4e36aeff-be3a-44cc-a78d-934d00ed43c2

Method	Results	Remarks	Reference
Closed cup method			dissemination site[16]

8.5.1 Short summary and overall relevance of the provided information on flammable liquids

The Flash Point of one of the submitted specimens of Tea Tree Oil was determined, in duplicate according to EC Method A.9 and using a Pensky-Martens, Closed Cup Flashpoint Apparatus equipped with an IP15C, -5 to 110°C Thermometer.

The results obtained were as follows :-

a) 56°C

b) 55°C

The Atmospheric Pressure at the time of the determinations was 1007 mbar. Hence, the Flash Point of the Tea Tree Oil, being the lowest temperature at which the vapour from the material ignites, is 55°C at 1007 mbar.

Based on data in REACH registration dossier a GLP-compliant study was carried out to determine the flash point of Tea Tree Oil. The study followed the requirements of EEC method A9 without significant deviation. The flash point of Tea Tree Oil was found to be 55.0°C and 54.0°C in duplicate tests using the equilibrium method and a Mensky-Martens closed tester, respectively.

8.5.2 Comparison with the CLP criteria

The substance has to be classified as flammable liquid in the category 3 according to the Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures (including all amendments) since its flash point is ≥ 23°C and ≤ 60°C.

8.5.3 Conclusion on classification and labelling for flammable liquids

The substance has to be assigned to the category 3 for flammable liquids. Labelling proposed is **H226-Flammable Liquid.**

[16] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/12

8.6 Flammable solids

Hazard class is not applicable (TTO is liquid and not a solid).

8.6.1 Short summary and overall relevance of the provided information on flammable solids

Hazard class not applicable: The substance is a liquid.

8.6.2 Comparison with the CLP criteria

Hazard class not applicable: The substance is a liquid.

8.6.3 Conclusion on classification and labelling for flammable solids

Hazard class not applicable: The substance is a liquid.

8.7 Self-reactive substances

Table 10: Summary table of studies on self-reactivity

Method	Results	Remarks	Reference
Differential Scanning Calorimetry	No significant exothermic events	-	Parsons, A. (2007)
EC A15- Autoflammability	269°C	-	Parsons, A. (2007)
EC A15- Autoflammability	252°C at 1020 mbar	-	ECHA dissemination site[17]

8.7.1 Short summary and overall relevance of the provided information on self-reactive substances

A sample of the product was subjected to Differential Scanning Calorimetry on a Perkin Elmer, Pyris 6 DSC. The sample was examined over the range 30°C to 400°C, programmed at a rate of 10°C/min and two replicate runs were carried out.

It was found that there were no significant exothermic events that occurred during this test, which would indicate that it is very unlikely that a thermally induced, explosive reaction is likely to occur with this material.

The testing was carried out with a modified, temperature programmed, Gas Chromatograph Oven. A Portec Type K, PI 8013, Electronic Thermometer and an RS Ltd., Digital Timer.

The following results were obtained :

The auto-ignition temperature of the specimen is 269°C, with a sample volume of 30µl, a delay time of 14 seconds and at a barometric pressure of 1008 mbar.Based on data in REACH registration dossier a GLP-compliant study was carried out to determine the auto-ignition temperature of Tea Tree Oil. The study followed the requirements of EEC method A15 without significant deviation. The auto-ignition temperature of Tea Tree Oil was found to be 252°C.

8.7.2 Comparison with the CLP criteria

The substance does not meet the CLP criteria for classification for this hazard class.

[17] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/13

8.7.3 Conclusion on classification and labelling for self-reactive substances

Not a self-reactive substance. Data is conclusive but not sufficient for classification.

8.8 Pyrophoric liquids

Table 11: Summary table of studies on pyrophoric liquids

Method	Results	Remarks	Reference
EC A.15- Autoflammability	269°C	-	Parsons, A. (2007)

8.8.1 Short summary and overall relevance of the provided information on pyrophoric liquids

The testing was carried out with a modified, temperature programmed, Gas Chromatograph Oven containing the flask etc. A Portec Type K, PI 8013, Electronic Thermometer and an RS Ltd., Digital Timer.

The following results were obtained :

The auto-ignition temperature of the specimen is 269°C, with a sample volume of 30µl, a delay time of 14 seconds and at a barometric pressure of 1008 mbar.

8.8.2 Comparison with the CLP criteria

The substance does not ignite within 5 min when added to an inert carrier and exposed to air, nor does it ignite or char a filter paper on contact with air within 5 min.

8.8.3 Conclusion on classification and labelling for pyrophoric liquids

The substance is no pyrophoric liquid. Data is conclusive but not sufficient for classification.

8.9 Pyrophoric solids

8.9.1 Short summary and overall relevance of the provided information on pyrophoric solids

Hazard class not applicable: The substance is a liquid.

8.9.2 Comparison with the CLP criteria

Hazard class not applicable: The substance is a liquid.

8.9.3 Conclusion on classification and labelling for pyrophoric solids

Hazard class not applicable: The substance is a liquid.

8.10 Self-heating substances

Table 12: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
EC A15- Autoflammability	269°C	-	Parsons, A. (2007)
EC A15- Autoflammability	252°C at 1020 mbar	-	ECHA dissemination site[18]

[18] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/4/13

8.10.1 Short summary and overall relevance of the provided information on self-heating substances

The auto-ignition temperature of the specimen is 269°C, with a sample volume of 30µl, a delay time of 14 seconds and at a barometric pressure of 1008 mbar.

Based on data in REACH registration dossier a GLP-compliant study was carried out to determine the auto-ignition temperature of Tea Tree Oil. The study followed the requirements of EEC method A15 without significant deviation. The auto-ignition temperature of Tea Tree Oil was found to be 252°C.

8.10.2 Comparison with the CLP criteria

The substance does not meet the criteria for classification for this hazard class.

8.10.3 Conclusion on classification and labelling for self-heating substances

Not a self-heating substance. Data is conclusive but not sufficient for classification.

8.11 Substances which in contact with water emit flammable gases

8.11.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

Based on the chemical structure of the substance and the experience in manufacture and handling, the substance does not react with water. Thus, a study does not need to be conducted according to Regulation (EC) No 1272/2008, Annex I, part 2 (2.12.4.1).

8.11.2 Comparison with the CLP criteria

Not required.

8.11.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

Hazard class not applicable.

8.12 Oxidising liquids

Table 13: Summary table of studies on oxidising liquids

Method	Results	Remarks	Reference
Reasoned case	No likely or realistic possibility of this substance being an Oxidation Hazard.	-	Parsons, A. (2007)

8.12.1 Short summary and overall relevance of the provided information on oxidising liquids

From a consideration of each of the individual ingredients of the substance there is no indication to suggest that either alone or in combination, they are likely to be an oxidation hazard.

As also all the ingredients are products of long standing which have been used on a large scale, with no recorded problems in respect of an oxidation hazard, it would suggest that it is extremely unlikely to pose any threat from this perspective.

The following points also apply when considering the possibility of this formulation constituting an Oxidation hazard:

a) In general, Oxidizing Properties are not expected if an organic molecule does not contain oxygen, chlorine or fluorine at all, or if, as in this case, the molecules of the major constituents of the Tea Tree Oil, either do not contain any oxygen or, if they do, that it is chemically bonded to carbon or hydrogen only.

b) None of the major ingredients of the Tea Tree Oil contains a chemical group that might indicate the potential presence of oxidizing properties (e.g. peroxide, chlorate, perchlorate, nitrate, bromate, chromate etc.)

c) For an organic chemical, a very important factor in deciding whether that system might be an oxidation hazard, is the so-called, 'Oxygen Balance'. This is the difference between the oxygen content of the actual compound(s) and that required to fully oxidise the Carbon, Hydrogen and other oxidizable elements present in it, to carbon dioxide, water, etc. If there is a large deficiency of oxygen present, as there is with all the major compounds present (i.e. Terpinen-4-ol, Cineole, alpha-Terpineol, gamma-Terpinene, p-Cymene, alpha-Terpinene), then the balance is said to be negative meaning it is less likely that this compound/mixture will be an oxidising agent.

d) In the Differential Scanning Calorimetry experiments carried out earlier in connection with the explosive properties, there were no indications of any significant reactions when the material was heated up to 400°C, which would again tend to indicate that the material was stable and unlikely to react readily with other materials and that as such was unlikely to form an oxidising hazard.

It is therefore our considered opinion from all the above, that there is no likely or realistic possibility of this material being an oxidation hazard and that therefore, the actual oxidation properties testing should not be required.

8.12.2 Comparison with the CLP criteria

The substance does not meet the criteria for classification for this hazard class.

8.12.3 Conclusion on classification and labelling for oxidising liquids

Not an oxidising substance. Data is conclusive but not sufficient for classification.

8.13 Oxidising solids

8.13.1 Short summary and overall relevance of the provided information on oxidising solids

Hazard class not applicable: The substance is a liquid.

8.13.2 Comparison with the CLP criteria

Hazard class not applicable: The substance is a liquid.

8.13.3 Conclusion on classification and labelling for oxidising solids

Hazard class not applicable: The substance is a liquid.

8.14 Organic peroxides

8.14.1 Short summary and overall relevance of the provided information on organic peroxides

Hazard class not applicable: The substance is not an organic peroxide.

8.14.2 Comparison with the CLP criteria

Hazard class not applicable: The substance is not an organic peroxide.

8.14.3 Conclusion on classification and labelling for organic peroxides

Hazard class not applicable: The substance is not an organic peroxide.

8.15 Corrosive to metals

8.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

No test data are available. However, based on the experience in manufacture and handling the substance does not materially damage metallic containers.

8.15.2 Comparison with the CLP criteria

Not relevant

8.15.3 Conclusion on classification and labelling for corrosive to metals

Hazard class not applicable.

8.16 Desensitized explosives

The formulation does not contain substances to suppress or reduce their explosive properties.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

No ADME studies were available for Tea Tree Oil

Table 14: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
No data for TTO			

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

No ADME studies were available for Tea Tree Oil. However, since the major part of the terpene components of TTO are commonly occurring compounds found in many plants, there is extensive data and publicly available literature for these components available. Thus, for the AIR-4 renewal process of TTO, no specific ADME study was provided for TTO in order to avoid further animal testing.

An extensive literature search for TTO and its single components was performed according to EFSA Journal 2011; 9(2):2092.

Several publications were found for the single terpene components of TTO, most for 1,8-Cineole, p-Cymene, d-Limonene, and α-Pinene. Few for α-terpineol, γ-Terpinene, Terpinen-4-ol and δ-Cadinene. During literature search, no study was found for Sabinene, Aromadendrene, Ledene, Globulol and Viridiflorol.

Besides being naturally present in plants, most of the terpene components of TTO were globally used as fragrances and flavouring additives in consumer products. The health effects and risk for consumers has already been assessed by the International Agency for Research and Cancer (IARC/WHO) and the European Food Safety Authority (EFSA) (for reference details please refer to ANNEX 2 of CLH report).

Thereby, terpenes were allocated to different chemical groups according to their chemical structure and risks were assessed for group members based on available data for supporting substances of similar chemical structure. For consumer risk assessment, information on ADME is crucial and was therefore also evaluated within these official documents (ANNEX 2 of CLH report).

For the purpose of characterization of ADME of TTO, these official evaluations serve as a base for the following assessment. Those terpenes which were not part of the evaluation by EFSA/WHO itself were allocated by the notifier to the respective chemical groups according to their chemical structure.

Due to the structural similarity to the already evaluated substances (same or no additional functional groups) no different metabolic pathways as described for the evaluated substances are expected. The following grouping was performed:

	Chemical group			
	Aliphatic and alicyclic hydrocarbons	**Aromatic hydrocarbons**	**Aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances**	**Alicyclic ethers**
Terpenes evaluated under WHO/EFSA	monocyclic • d-Limonene • α-Terpinene • γ-Terpinene • α-Terpinolene bicyclic • α-Pinene	• p-Cymene	• α-terpineol • Terpinen-4-ol	• 1,8-Cineole

	Chemical group			
	Aliphatic and alicyclic hydrocarbons	**Aromatic hydrocarbons**	**Aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances**	**Alicyclic ethers**
	• δ-cadinene			
Terpenes grouped according to their chemical structure	• Sabinene • Aromadendrene • Ledene	--	• Globulol • Viridiflorol	--
Reference	EFSA J. 4053 EFSA J. 4067 EFSA J. 931 EFSA J. 918 WHO TRS 928	EFSA J. 931	WHO TRS 891	WHO TRS 922 EFSA J. 639, EFSA J. 3092

Additionally to those information provided by EFSA/WHO, further peer reviewed literature studies, which were not used within the EFSA/WHO evaluations, were assessed to broaden information on ADME of the TTO terpene components. A large number of open literature studies is available. Main focus was set on absorption, distribution, elimination and identification of metabolites in animals and humans. Furthermore, special focus was also set on inhalative absorption since TTO and the vast majority of its components were highly volatile

In the following, an overview table is given for ADME of the different terpenes belonging to the above mentioned different chemical groups.

Table 15: Toxicokinetic properties of TTO components

Chemical class of terpenes	Terpenes evaluated within the chemical group	Absorption, Distribution and Excretion	Metabolism
Aliphatic and alicyclic hydrocarbons	<u>Monocyclic:</u> • d-Limonene • α-Terpinene • γ-Terpinene • α-Terpinolene <u>Bicyclic:</u> • α-Pinene • δ-Cadinene • Sabinene <u>Tricyclic:</u> • Aromadendrene • Ledene	Being lipophilic, the aliphatic and alicyclic hydrocarbons in this group are likely to cross biological membranes by passive diffusion. After oral, dermal and inhalation exposure, they are rapidly absorbed, distributed to lipophilic body tissues and extensively metabolized. Elimination from blood follows a triphasic pattern, with a slow terminal phase. Elimination occurs mainly via urinary excretion in the form of conjugated polar metabolites; only small amounts are excreted via faeces or by exhalation.	On the basis of the available data, it is anticipated that all the aliphatic and alicyclic hydrocarbons in this group will participate in similar pathways of metabolic detoxification in mammals, including humans. After absorption, these hydrocarbons are oxidized to polar oxygenated metabolites via cytochrome P450 (CYP) enzymes and alcohol and aldehyde dehydrogenases. The aliphatic and alicyclic substances are oxidized either by side-chain oxidation or by epoxidation of an exocyclic or endocyclic double bond. Alkyl oxidation initially yields hydroxylated metabolites that may be excreted in conjugated form or undergo further oxidation, yielding more polar metabolites that are also excreted in conjugated form in the urine. If a double bond is present,

Chemical class of terpenes	Terpenes evaluated within the chemical group	Absorption, Distribution and Excretion	Metabolism
			epoxide metabolites may form and these metabolites are detoxified either by hydrolysis to yield diols, or by conjugation with glutathione.
Aromatic hydrocarbons	• p-Cymene	p-Cymene is rapidly and well absorbed following oral and dermal administration, or exposure by inhalation. After absorption, p-Cymene was immediately distributed in body tissues. Excretion was rapidly and nearly complete within 48 h.	*p*-Cymene undergoes extensive oxidation of the methyl substituent and isopropyl side-chain to yield polar oxygenated metabolites. Oxidation on the carbons of the benzene ring was seldom. A large percentage of the urinary metabolites was conjugated both to glucuronic acid and glycine.
Aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances	• α-Terpineol • Terpinen-4-ol • Globulol • Viridiflorol	Alicyclic tertiary terpenoid alcohols are rapidly absorbed after oral administration and inhalation exposure. In humans and animals, terpenoid tertiary alcohols are conjugated with glucuronic acid and are excreted in the urine and faeces	Unsaturated terpenoid alcohols may undergo allylic oxidation to form polar diol metabolites, which may be excreted either free or conjugated. If the diol contains a primary alcohol function, it may under-go further oxidation to the corresponding carboxylic acid. Metabolic oxidation is mediated by CYP450 enzymes.
Alicyclic ethers	• 1,8-Cineole	In humans and animals, 1,8-Cineole is rapidly absorbed after oral and inhalation exposure, extensively metabolized and eliminated by conjugation to polar metabolites. Elimination occurs in a biphasic pattern within a few hours. There are no indications for tissue accumulation. 1,8-Cineol is further excreted by exhalation. 1,8-Cineole and its metabolites are able to cross the blood-milk-barrier.	In humans and animals, 1,8-Cineole, has been shown to be oxidised via P450 isoenzymes to yield polar hydroxylated metabolites, which are conjugated and excreted or further oxidised and excreted. 1,8-Cineole principally undergoes ring-hydroxylation to form amongst others 2-or 3-hydroxy-1,8-Cineole. Cleavage of the ether is, at most, a very minor metabolic pathway.

***ADME Summary*:**

- Based on ADME data of TTO constituents, TTO is metabolized and excreted from experimental animals within 2-3 days, mainly via urine (d-limonene in Wistar rats cleared within 48 hours).
- There is no evidence of bioaccumulation due to major biotransformation reactions taking place in the liver and to a lesser extent in other organs.
- Due to the structural similarity to the already evaluated substances (same or no additional functional groups) no different metabolic pathways as described for the evaluated substances are expected; metabolism of the components is comparable. Therefore it can be concluded that no dangerous/non-toxic metabolites are synthetized.

10 EVALUATION OF HEALTH HAZARDS

For details of the summarized studies on health hazards of Tea Tree Oil, please refer to Annex 2 of the CLH report.

10.1 Acute toxicity - oral route

The acute oral toxicity of Tea Tree Oil has been assessed in rats.

Table 16: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels, duration of exposure**	**Value LD_{50}**	**Reliability score**	**Reference**
Acute oral toxicity study in rats OECD 425 (2008) (Acute Oral Toxicity Up-and-Down Procedure) GLP	Rat, Wistar Hsd Han: females, 3/group	Tea Tree Oil 9.7% α-Terpinene, 1.5% p-Cymene, 2.6% 1.8-Cineol, 17.8% γ-Terpinene and 41.5% Terpinen-4-ol (complies with ISO specifitaion)	550 mg TTO/kg (Group 1) and 2000 mg TTO/kg (Group 2) 14 days	1049 mg/kg bw <u>Clinical signs:</u> 550 mg TTO/kg: no clinical signs or mortality 2000 mg TTO/kg: Hypoactivity, slight tremors recumbeny, death on day 1 – 2 after dosing.	1	Anonymous 2015a
Acute oral toxicity of Tea Tree Oil in the rat OECD 401 GLP not stated	Rat, Sprague Dawley SPF rats - Specific Pathogen-free and non-SPF-rats males, females, 5/group	Tea Tree Oil (no information on composition available)	3, 2.75, 2.6 and 2.5 mL/kg bw (SPF rats – Specific Pathogen-Free) and 2.4, 2.25, 2.15, 2.10 and 1.70 mL/kg bw (non-SPF rats) 14 days	2.6 mL/kg bw in SPF rats 1.9 mL/kg bw (≈ 1682 - 1721 mg/kg bw) in non-SPF rats <u>Clinical signs:</u> Surviving SPF and non-SPF rats: lack of tonus in the forelimbs, weeping eyes, bloodied noses.	2	Anonymous 1989a and ECHA disseminati on site[19]
Acute oral toxicity in oral: gavage OECD Guideline 423; EU Method	mouse (CRL:(NMRI) BR Mouse) female 3/group	Melaleuca alternifolia, ext., purity: 100% (complies with ISO	2000mg TTO/kg bw (Group 1) and 2000 mg TTO/kg bw (Group 2) Observation	LD_{50}: >2000 mg/kg bw (female) based on: (test mat.)	1	ECHA disseminati on site[20]

[19] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/3/2

[20]https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/3/2/?documentUUID=77b26afa-3b23-4d8a-a1a7-7c769040fa3c

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reliability score	Reference
B.1; EPA OPPTS 870.1100 GLP		4730:2017specifitaion)	period: 14d			

Literature studies

Open scientific literature search has been performed and some data on the acute oral toxicity of the Tea Tree Oil components have been found. The toxicity of tested monoterpenes is found to be comparable.

The following table presents all the data found for the individual components.

Table 17: Summary table of other studies relevant for acute oral toxicity

Type of study/data	Test substance	Observations	Reliability score	Reference
Acute oral / Rat	1,8-Cineole	LD_{50} = 1280 mg/kg > 100 mg/kg: Tremor, convulsion, abnormal gait and ataxia, increased respiration, decreased activity, unresponsiveness to writhing test, flaccid paralysis (leading to recumbencey). <u>Mortality latency:</u> 1000 mg/kg: >6, <15 h 1600 mg/kg: >2, <10 h 2900 mg/kg: >2, <3 h <u>Intestinal transit:</u> 20-120 mg/kg: Slight and non-significant decrease in traversing in the small intestine.	2	Jalilzadeh-Amin *et al.* (2015)
Acute oral / Mice	1,8-Cineole	LD_{50} = 3849 mg/kg Lethal dose: Rapid cyanosis, stupor, irregular breathing, extreme sensitivity to noise and convulsions. 21.38 and 64.15 mg/kg: Central venous congestion of liver lobule, granular degeneration of hepatocytes. 192.45 mg/kg: Central venous congestion, granular degeneration, vacuolar degeneration and hepatic necrosis; distorted and fractured endoplasmic reticulum, ribosomes scattered into cytoplasm, swollen mitochondria with disorganized cristae. (liver, kidney) 64.15 and 192.45 mg/kg: Capillary of glomerulus and interstitial angiectasis hyperemia, renal tubular epithelial cells swelling, granular degeneration and partially separating from basement membrane, amount of eosinophilic protein exudation existing in tubular lumen	2	Xu *et al.* (2014)
Acute oral / Rat	1,8-Cineole	1500 mg/kg < LD_{50} < 1750 mg/kg <u>Single dosing:</u> 1500 mg/kg: sedation, tremor, significant increase in consumption of food, water and body weight.	2	Caldas *et al.* (2016)

Type of study/data	Test substance	Observations	Reliability score	Reference
		1750 and 2000 mg/kg: sedation, tremor, diarrhea, difficulty breathing and seizures, death within less than 24 h. Repeated dosing: 500 and 1000 mg/kg: diarrhea and reduced body weight during the first week, followed by an increase until the end of treatment. Hematology and biochemistry: 500 and 1000 mg/kg: Significant increase of MCV and decrease in MCHC and MPV (males); increase in the level of urea (females). 100 mg/kg: Decrease in the level of alkaline phosphatase (males). Morphology: 500 and 1000 mg/kg: Decrease in absolute weigth of the lungs and spleen (males), lymphocytic infiltrate in the liver (females). 1000 mg/kg: increase in absolute and relative weight of liver (females), increase of glomerular space in kidneys. All doses: Eosinic and lymphocytic infiltrate in the lungs (males and females), in the liver (males) and into the uterus (females). Reproductive toxicity: All doses: Significant decrease in maternal weight gain during pre-implantation and organogenesis. 1000 mg/kg: Significant decrease in maternal weight gain during pregnancy, dead fetuses and reduction of the mass of fetuses. 250 mg/kg: Reduction in the number of corpora lutea.		
Acute oral / Rat, Mice	γ-Terpinene	LD_{50} > 2000 mg/kg No sign of evident toxicity, no behavioural and clinical alterations. 12.5 and 25 mg/kg: Significant reduction of the licking time of pain-stimulated paw. ≤ 6.25 mg/kg: Significant inhibition of glutamate-induced nociception.	2	de Brito Passos *et al*. (2015)
Acute oral / Rat	Terpinen-4-ol (4-Carvomenthenol)	LD_{50} = 1300 mg/kg	4	RIFM Report number 1695 (1977)**
Acute oral / Rat	γ-Terpinene	LD_{50} = 3650 mg/kg	4	Moreno (1973b)*
Acute oral / Rat	α-Terpinene	LD_{50} = 1680 mg/kg	4	Moreno (1973a)*
Acute oral / Rat	α-Pinene	LD_{50} = 3700 mg/kg	4	Moreno (1972e*)

Type of study/data	Test substance	Observations	Reliability score	Reference
Acute oral / Rat	p-Cymene	LD_{50} = 4750 mg/kg	4	Jenner (1964)*
Acute oral / Rat	α-Terpinolene	LD_{50} = 3784 mg/kg	4	Brownleer (1940)*

*Cited in T.B. Adams et al./Food and Chemical Toxicology 49 (2011)2471-2494. The studies are published and were not available for reliability assessment.
** Cited in S. P. Bhatia *et al*. (2008), Food and Chemical Toxicology 46 (2008) 91-94.

Reliability statement: The literature studies from which the data listed in the above table have not been performed according to official test guidelines. Therefore, it is not possible to evaluate their compliance by identifying any deviations or comparing them against guideline-specific quality criteria. Nonetheless, the publications in question have been assessed for relevance and scientific reliability according to the criteria set out in the EFSA guidance for submission of scientific literature (EFSA Journal 2011;9(2):2092) and assigned a respective reliability score. Details of this process are described in the literature section (CA 9) of the active substance dossier, which clarifies that all studies used in the dossier were classified by the applicant as reliable (reliability score: 1) or reliable with restrictions (reliability score: 2, supporting information). Studies with reliability score 3 (not reliable) are only presented in section 11 (aquatic ecotoxicity). Studies attributed with a reliability score of 4 (not assignable) were not available for reliability assessment and are thus not relied upon.

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

Study 1, Anonymous 2015a, Tea Tree Oil: Acute oral toxicity study (Up-and-Down Procedure) in Wistar rats, OECD 425, GLP.

Reliability statement: The study is conducted in accordance with OECD 425. It meets the validity requirements as set out in the test guideline, shows only minor deviations and the test substance reflects the ISO 4730:2004. Hence, the study is considered reliable without restictions (reliability score: 1).

In the 2015 study, Tea Tree Oil, was tested for its potential acute hazard after a single oral administration at a dosage volume of 0.13 mL resulting in a target dose of 550 mg/kg for the group 1 and approx. 0.47 mL resulting in a target dose of 2000 mg/kg for the group 2. Six female Wistar rats were dosed sequentially by gavage, each group at 3 steps at a minimum of 48 hours as follow:

Each of the six female Wistar rats were sequentially dosed by gavage with Tea Tree Oil at a target dose as follow:

No mortality and clinical signs were observed in all the animals dosed with 550 mg TTO/kg bw (Group 1) throughout the entire 14-day observation period.

The rats dosed with 2000 mg TTO/kg bw (Group 2) exhibited clinical signs such as hypoactivity and slight tremors and died on day 1 or day 2.

All the survived rats gained weight during the 14-day observation period. The pre-terminal dead rats lost weight when compared to their initial body weight.

Based on the results obtained, the estimated acute oral LD_{50} of Tea Tree Oil is 1049 mg/kg bw. with 95% confidence interval of 550 to 2000 mg/kg body weight in female rats.

In accordance with the EC Directives on dangerous preparations 1272/2008, Tea Tree Oil is classified as acute oral Category 4 ($300 < ATE \leq 2000$).

Study 2, Anonymous 1989a, Acute oral toxicity of Tea Tree Oil in the rat, OECD 401.

Reliability statement: The study was accepted during previous evaluation and is conducted in accordance with OECD 401. However, it shows some deviations in terms of methodology and reporting, which were not deemed to have an influence on its overall scientific reliability. More deviations would become obvious when comparing the study to the current test guidelines for oral toxicity studies, which show a significantly altered design. Overall, the study is therefore considered reliable with restrictions (reliability score: 2).

The acute oral toxicity of Tea Tree Oil was assessed according to the OECD Guideline no. 401.

Groups of 5 male and 5 female Sprague Dawley rats weighing between 146 and 219 grams received a single oral dose of 3, 2.75, 2.6 and 2.5 mL/kg bw (SPF rats – Specific Pathogen-Free) and 2.4, 2.25, 2.15, 2.10 and 1.70 mL/kg bw (non-SPF rats) as a suspension in peanut oil. The samples were diluted with peanut oil w/w at 3 different concentrations: 1/3, 1/4 and 1/5. The animals were fed on a diet of rat and mouse cubes and tap water *ad libitum*, and fasted for 24 hrs prior to treatment. The animals were observed during the experimental period of 14 days after treatment for mortality and signs of toxicity.

The LD_{50} for mortality was found to be 2.6 mL/kg bw in SPF rats and 1.9 mL/kg bw (≈ 1682 - 1721 mg/kg bw) in non- SPF rats, respectively.

Surviving animals showed lack of tonus in the forelimbs, weeping eyes and bloodied noses.

Based on data on ECHA dissemination site a GLP-compliant study was conducted in accordance with OECD Guideline 423 (acute toxic class method) to determine the acute oral toxicity of Tea Tree Oil to female CRL:(NMRI)BA mice. In this two-step study, three animals were dosed in the initial step with Tea Tree Oil (formulated in PEG 400) at a dose level of 2000 mg/kg bw, followed by an observation period of 14 days. In the absence of any mortailities, a confirmatory group of three animals was then tested at the same dose level. There were no mortalities or macroscopic findings related to treatment and no clear indications of effects on bodyweight. Clinical signs included decreased activity, hunched back position, uncoordination, piloerection, decreased grip reflex, decreased respiratory rate and/or dyspnoea, none of which persisted beyond day 8 of treatment. In conclusion, under the conditions of this study, the acute oral LD_{50} of Tea Tree Oil was > 2000 mg/kg bw, when administered to female mice.

The literature studies show that the individual components of Tea Tree Oil do not lead to lower LD_{50} values and therefore support the allocation of Tea Tree Oil into the category 4 of the acute toxicity hazard.

10.1.2 Comparison with the CLP criteria

In accordance with the Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures (including all amendments, Tea Tree Oil is classified as acute oral Category 4 ($300 < ATE \leq 2000$).

10.1.3 Conclusion on classification and labelling for acute oral toxicity

TTO is proposed to classify as Acute oral Category 4with hazard statement **H302- Harmful if swallowed**. Proposed $ATE_{oral\ acute}$ = 1049 mg/kg bw/d.

10.2 Acute toxicity - dermal route

The acute dermal toxicity of Tea Tree Oil was tested in male and female rats and rabbits. The new study from 2015 was conducted with rats.

Table 18: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Value LD_{50}	Reliability score	Reference
Tea Tree Oil: Acute dermal toxicity study in Wistar rats OECD 402 (1987) GLP	Rats, Wistar male, female, 2 groups, 5/sex	Tea Tree Oil 9.7% α-Terpinene, 1.5% p-Cymene, 2.6% 1.8-Cineol, 17.8% γ-Terpinene and 41.5% Terpinen-4-ol (complies with ISO-specification)	Undiluted test item, 2000 mg/kg bw (2.24 ml/kg bw) 24 hours	> 2000 mg/kg bw No clinical signs occurred.	1	Anonymous 2015b
Acute dermal toxicity limit test of Tea Tree Oil batch 88/375 in the rabbit OECD 402 GLP not stated	Rabbit, New Zealand White 5 males and 5 females	Tea Tree Oil (no information on composition available)	Undiluted test item, 2000 mg/kg bw 24 hours	> 2000 mg/kg bw Clinical signs: Slight diarrhoea in 1/10 animals.	2	Anonymous 1989b and ECHA dissemination site[21]

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

Study 1, Anonymous 2015b, Tea Tree Oil: Acute dermal toxicity study in Wistar rats, OECD 402 (1987), GLP.

Reliability statement: The study was conducted according to the former version of OECD 402. The design and methodological structure of the updated OECD guideline 402 (2017) differs in part significantly from the version used and was not yet in place at the time of study conduction. Due to these differences, a comparison of the study with the current guideline would inevitably lead to the identification of number of inherent deviations, which would thus be of limited informative value and could give a distorted picture of its reliability. Therefore, the study is considered reliable without restrictions (reliability score: 1)

In the new study from 2015, the acute dermal toxicity of Tea Tree Oil was tested in male and female Wistar rats.

Based on the individual body weights, the undiluted test item at dose of 2000 mg/kg bw. (2.24 mL/kg bw.) was applied directly to the clipped skin of the animal to cover about 10% of the body surface of the animal. The applied area was covered with cotton gauze. The test item contact period with the skin was for 24 hours.

After the 24 hour contact period, the dressing was removed and the applied area was washed with water.

All the rats were observed for clinical signs of toxicity and mortality for 14 days post application. At the end of the observation period, all animals were euthanized and subjected to necropsy.

[21] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/3/4

There were no clinical signs and mortality observed. All rats gained weight during the experimental period. No abnormalities detected at the necropsy.

Study 2, Anonymous 1989b, Acute dermal toxicity limit test of Tea Tree Oil batch 88/375 in the rabbit, OECD 402.

Reliability statement: The study was largely conducted according to the former version of OECD 402 and accepted during previous evaluation. The design and methodological structure of the updated OECD guideline 402 (2017) differs in part significantly from the version used and was not yet in place at the time of study conduction. Due to these differences, a comparison of the study with the current guideline would inevitably lead to the identification of number of inherent deviations, which would thus be of limited informative value and could give a distorted picture of its reliability. However, the study reveals some considerable deviations also from the former guideline in terms of methodological and reporting deficiencies. Yet, these were not deemed to have an influence on its overall scientific reliability. Therefore, the study is overall considered reliable with restrictions (reliability score: 2).

In support, in the 1989 study (pre-GLP), the undiluted test sample was applied dermally at a dose of 2000 mg/kg bw and held in contact with the skin for 24 hours over an approximate skin area of 175 cm^2.

The animals were then observed during the 24 hour exposure period and daily for 14 days thereafter. Observations were made for any signs of toxicity and abnormal behavior. The body weight was determined on days 0, 7 and 14. No mortality was observed and there were no other signs of toxicity or abnormal behaviour. No significant loss of weight was observed during the observations period.

10.2.2 Comparison with the CLP criteria

Based on the results obtained, the estimated acute dermal LD_{50} of Tea Tree Oil is above 2000 mg/kg bw for male and female rats as well as male and female rabbits. The application on the guidance of the CLP criteria (Regulation (EC) 1272/2008) gives a cut off LD_{50} value of 2000 mg/kg bw for acute dermal toxicity classification.

Therefore, Tea Tree Oil does not require to be classified for the acute dermal toxicity.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Not classified. Data conclusive but not sufficient for classification.

10.3 Acute toxicity - inhalation route

The acute toxicity via the inhalation route with Tea Tree Oil was assessed with Wistar rats.

Table 19: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, form and particle size (MMAD)**	**Dose levels, duration of exposure**	**Value LC_{50}**	**Reliability score**	**Reference**
Tea Tree Oil: Acute Inhalation Toxicity Study in Wistar Rats OECD 403 (2009)	Rats, Wistar HsdCpb: WU males, females, 5 per group	Tea Tree Oil, 9.45 % α-Terpinene, 5.67 % 1,8-Cineole, 21.04 % γ-Terpinene, 2.35 % p-cymene and 37.98 % Terpinen-4-ol aerosol (complies with ISO-	0.77, 3.69 and 5.06 mg TTO/L of chamber air Continuous exposure for 4 hours	3.64 mg/L air (4 h, male & female rats) Clinical signs: Please refer to Table 20	1	Anonymous. 2010a

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reliability score	Reference
GLP		specification)				
Acute Inhalation Toxicity Study in Wistar Rats Inhalation: aerosol (nose only) OECD 403 GLP	Rats, Wistar CRL:(WI) BR males, females, 5 per group	Melaleuca alternifolia, ext., purity: 100% (complies with ISO 4730:2017specifitaion) MMAD: 2.31 - 3.51µm GSD: 2.05 – 2.42	1.94, 3.70, 5.04 mg/L Duration of exposure: 4 h	LC_{50}: 5.23 mg/L air (male) LC50: 4.29 mg/L air (female) LC50: 4.78 mg/L air (male/female)	1	ECHA dissemination site[22]

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

Study 1, Anonymous 2010a, Tea Tree Oil: Acute Inhalation Toxicity Study in Wistar Rats, OECD 403 (1987), GLP.

Reliability statement: The study is conducted in accordance with OECD 403. It meets the validity requirements as set out in the test guideline, shows only minor deviations and the test substance reflects the ISO 4730:2004. Hence, the study is considered reliable without restictions (reliability score: 1).

The aim of this study was to assess the possible inhalation toxicity potential of Tea Tree Oil. The acute inhalation toxicity study with Tea Tree Oil was conducted in male and female Wistar rats by nose only exposure using 30%, 50% and 70% w/v aerosol of the test item diluted in Dimethyl sulphoxide to 3 groups of rats (G2, G3 and G4). The aerosol was generated by a glass atomizer with an injection rate of 0.4 mL/min. Similarly rats in the vehicle control group (G1) were exposed to Dimethyl sulphoxide aerosol only. The rats were continuously exposed to the test item aerosol for 4 hours in an inhalation exposure chamber. The post-treatment observation period was 14 days.

Mortality and clinical signs were observed immediately after exposure and thereafter once daily during days 2 to 15. Body weights were determined during acclimatization, on Day 1, 3, 8, 15 and at death. Macroscopic examination was performed after terminal sacrifice (Day 15).

Table 20: Clinical signs and mortality after exposure of TTO in an Acute Inhalation Study in *Wistar Rats* (a total of 10 animals per dosing group)

Test Item Concentration [% w/v]	Dose level		Toxic Signs [No. of incidences]										Mortality [%]	Necropsy findings [%]	Body weight
	mgTTO/L air		Ataxia	Dispnoea	Dullness	Lethargy	Nasal discharge	Perineum wet with urine	Recumbency	Slight salivation	Tremor	Death			

[22] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/3/3

Vehicle control (DMSO)	**0**		-	-	-	-	-	-	-	-	-	-	0	0	+
30 % w/v in DMSO	0.77	Day 1				10	10				1		10	0	+**
		Day 2			3	9									
		Day 3			1	1						1			
		Day 4*													
50 % w/v in DMSO	3.69	Day 1	1	1		10	8	2	1	4	8		40	0	+**
		Day 2	1		8	9		1			1	1			
		Day 3			1	2						3			
		Day 4*													
70 % w/v in DMSO	5.06	Day 1	6	8		2	9	1		5	3	1	70	0	+**
		Day 2		4	7	7						2			
		Day 3			1	4						3			
		Day 4*										1			

*: All surviving animals were normal from day 4 onwards;

**: Pre-terminally dead animals lost body weight when compared to its initial body weight

The test item concentration in the air inhalation sample columns were analyzed using a validated analytical method. The analytical determined mean test item concentrations in the air inhalation sample columns were 0, 0.77, 3.69 and 5.06 mg TTO/L of chamber air.

No mortality occurred in the control group G1. Mortality of 10, 40 and 70% occurred in G2, G3 and G4 groups, respectively without sex preference.

The acute inhalation LC_{50} (4 h) of Tea Tree Oil in Wistar rats was established to be 3.64 mg/L of air for both male and female rats.

Based on data on ECHA dissemination site a GLP-compliant study was carried out to determine the acute inhalation toxicity of Tea Tree Oil to rats. The study followed the requirements of OECD guideline 403, without significant deviation. Three groups of ten Wistar rats (five males and five females) were exposed to an aerosol atmosphere. The animals were exposed for a single four-hour period using a nose-only exposure system, followed by a fourteen day observation period. Seven mortalities (2/5 males, 5/5 females) occurred at the highest test concentration; a specific cause of death was not clearly determined. No mortalities occurred at the two lower test concentrations. The surviving males from Group 1 and the majority of surviving males from Group 3 showed bodyweight loss during the first week of the observation period. Necropsy of the surviving animals on completion of the fourteen day observation period did not reveal any test item-related gross findings up to a concentration of 5.04 mg/L. The acute inhalation median lethal concentrations (4-hr LC_{50}) and 95% confidence limits of Tea Tree Oil in rats were calculated to be:

- Male & Female : 4-hr $LC_{50:}$ - 4.78 (3.94 - 5.32) mg/L

- Male only : 4-hr LC_{50} : 5.23 mg/L

- Female only (4-hr LC_{50}: 4.29 (3.41 - 6.41) mg/L.

10.3.2 Comparison with the CLP criteria

The acute inhalation LC_{50}, 4h value of Tea Tree Oil in Wistar rats was established to be 3.64 mg/L of air for both male and female rats.

Based on these results Tea Tree Oil (Anonymous 2010a, ECHA dissemination site) has to be classified as category 4 ($1.0<LC_{50}\leq5.0$) with regard to the acute inhalation toxicity according to the Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures (including all amendments). Proposed $ATE_{inhalation\ acute}$ = 3.64 mg/L.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Classification

Harmonised classification proposed. Tea Tree Oil has to be classified as acute toxicity hazard category 4 when regarding inhalation exposure. Labelling proposed is **H332- Harmful if inhaled**. Proposed $ATE_{inhalation\ acute}$ = 3.64 mg/L.

10.4 Skin corrosion/irritation

The potential of TTO to induce skin corrosion/irritation was tested in two rabbit studies.

Table 21: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reliability score	Reference
Tea Tree Oil: Acute dermal irritation / corrosion study in New Zealand White rabbits OECD 404 (2002) GLP	New Zealand White rabbits males one rabbit for the initial test and two rabbits for the confirmatory test	Tea Tree Oil 9.7% α-Terpinene, 1.5% p-Cymene, 2.6% 1.8-Cineol, 17.8% γ-Terpinene and 41.5% Terpinen-4-ol (complies with ISO specification)	0.5 ml of Tea Tree Oil 4 hours contact time	Mean score reactions, indicating that the test item is a "moderate-irritant" according to Draize´s evaluation method: Mean scores for individual animals at 7 observation times (values reported as sum of erythema/edema per number of observation dates): Rabbit 1: Mean24-72h score for erythema 2.67; Mean 24-72h score for edema 1.00 Rabbit 2: 2.00; 1.00 Rabbit 3: 2.00; 1.00 Clinical signs such as scale formation and peeling / desquamation were observed in all the rabbits. The reaction was reversible after 7 day.	1	Anonymous 2015c
Acute dermal irritation in the rabbit of Tea Tree Oil batch 88/375. No OECD Guideline GLP not stated	New Zealand White albino rabbits 6 young and mature animals	Tea Tree Oil	Undiluted test item	Irritation reactions were observed on both intact and abraded skin after the treatment with the test item. The primary irritation index was found to be 5.0. Evaluation of the skin reactions according to the EU criteria revealed that the test material produced mean irritation scores of 3.08 and 1.83 for erythema and oedema for intact skin and mean irritation scores of 3.25 and 2.0 for erythema and oedema for abraded skin, respectively.	2	Anonymous 1989c

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance</th><th>Dose levels duration of exposure</th><th>Results
-Observations and time point of onset
-Mean scores/animal
-Reversibility</th><th>Reliability score</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td></td><td>Mean Scores for individual animals at 2 observation times (24 and 72
<table>
<tr><th rowspan="2">Animal No.</th><th colspan="2">Intact Skin</th><th colspan="2">Abraded Skin</th></tr>
<tr><th>Erythema</th><th>Edema</th><th>Erythema</th><th>Edema</th></tr>
<tr><td>1</td><td>2.5</td><td>1.5</td><td>2.5</td><td>1.5</td></tr>
<tr><td>2</td><td>3.0</td><td>2.0</td><td>3.0</td><td>2.0</td></tr>
<tr><td>3</td><td>3.5</td><td>2.0</td><td>3.5</td><td>2.0</td></tr>
<tr><td>4</td><td>3.5</td><td>2.0</td><td>3.5</td><td>2.5</td></tr>
<tr><td>5</td><td>3.0</td><td>2.0</td><td>3.5</td><td>2.0</td></tr>
<tr><td>6</td><td>3.0</td><td>1.5</td><td>3.5</td><td>2.0</td></tr>
</table></td><td></td><td></td></tr>
</table>

Literature studies

Open scientific literature search has been performed and a study on skin irritation of the Tea Tree Oil components have been found.

Reliability statement: The literature studies from which the data listed in the table below are derived have not been performed according to official test guidelines. Therefore, it is not possible to evaluate their compliance by identifying any deviations or comparing them against guideline-specific quality criteria. Nonetheless, the publications in question have been assessed for relevance and scientific reliability according to the criteria set out in the EFSA guidance for submission of scientific literature (EFSA Journal 2011;9(2):2092) and assigned a respective reliability score. Details of this process are described in the literature section (CA 9) of the active substance dossier, which clarifies that all studies used in the dossier were classified by the applicant as reliable (reliability score: 1) or reliable with restrictions (reliability score: 2, supporting information).

Table 22: Summary table of other studies relevant for skin corrosion/irritation

<table>
<tr><th>Type of study/data</th><th>Test substance</th><th>Relevant information about the study (as applicable)</th><th>Observations</th><th>Reliabilty score</th><th>Reference</th></tr>
<tr><td>Literature study

Correlations of the components of Tea Tree Oil with its antibacterial effects and skin irritation</td><td>TTOs were isolated from the leaves (TTO-L), twigs, and branches of M. alternifolia by steam distillation, and the components analyzed by gas chromatography–mass spectrometry. Results showed that components of TTO-L satisfied the International Organization for</td><td>Draize skin irritation assay (female 8-12 week old Wistar rats, 5 animals per test concentration)

Chronic liver toxicity : 3 animals

TTO test</td><td>In the Draize skin irritation assay TTO-L, did not cause significant skin irritation at 2.5 % per site.

At concentrations of 5% and 10% TTO irritating effects were seen:
<table>
<tr><th rowspan="2"></th><th colspan="2">Draize score</th></tr>
<tr><th>5 % TTO</th><th>10 % TTO</th></tr>
</table></td><td>2</td><td>Lee, C.-J., Chen, L.-W., Chen, L.-G., Chang, T.-L., Huang, C.-W., Huang, M.-C., Wang, C.-C.; 2013</td></tr>
</table>

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reliabilty score	Reference
Reliable with restrictions Supportive information •No OECD guideline or GLP defined	Standardization (ISO) 4730 guidelines. Yields:TTO_{Leaves} = 2.2% TTO_{Twigs} = 0.59 % $TTO_{Branches}$ = 0.01 % Major components in TTO_{Leaves}:Terpinen-4-ol: 47.3%γ-terpinene: 20.59%α-terpinene: 9.58%1,8-cineole: 1.71%	concentrations: 0.625 %, 1.25 %, 2.5 %, 5 %. Vehicle not specified for sensitization test. In other parts of the study: dilution in jojoba oil.	**After 0 h** Edema 0 0 Erythema 0 0 **After 24 h** Edema 1 1 Erythema 1 2 **After 48 h** Edema 1 1 Erythema 1 2 Terpinen-4-ol did not cause skin irritation at up to 1.5%, whereas 1,8-cineole induced skin irritation in female *Wistar Rats* (8-10 weeks old) at dose rates of 0.75% and 1.5% but not at 0.375% and lower concentrations.		

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

Study 1, Anonymous, 2015c, Tea Tree Oil: Acute dermal irritation / corrosion study in New Zealand White rabbits, OECD 404 (2002), GLP.

Reliability statement: The study is conducted in accordance with OECD 404. It meets the validity requirements as set out in the test guideline, shows only minor deviations and the test substance reflects the ISO 4730:2004. Hence, the study is considered reliable without restictions (reliability score: 1).

In the 2015 study, the acute dermal irritation with New Zealand White rabbits was performed to evaluate the skin irritation potential of Tea Tree Oil.

A volume of 0.5 mL of undiluted test item was applied in between the prepared area of the skin and the cotton gauze of approx. size of 6 cm^2. A control patch was applied 3 – 4 cm anterior to the 4 hour test patch. All the patches were secured to the body of the animals by an adhesive tape, and a crepe bandage (except for 3 min patch) was wrapped around the torso of the animal. After a contact period of 4 hours, the treated are was washed with de-ionised water. The study was conducted in a stepwise manner (i.e., one rabbit for the initial test and two rabbits for the confirmatory test).

The degree of irritation was evaluated and scored by Draize´s evaluation method (1959) at 1, 24, 48 and 72 hours and day 7 and 14 post removal of the test patch.

The mean score reactions from gradings at 24, 48 and 72 hours after patch removal calculated for each individual animal were 2.67, 2.00, 2.00 for erythema and 1.00, 1.00, 1.00 for oedema,. Clinical signs such as scale formation and peeling / desquamation were observed in all the rabbits were reversible after 7 day observation. There were no pre-terminal deaths observed and no abnormality was detected at necropsy.

Study 2, Anonymous., 1989c, Acute dermal irritation in the rabbit of Tea Tree Oil batch 88/375.

Reliability statement: Although no GLP-study, it was largely conducted according to the former version of OECD 404 and accepted during previous evaluation. The design and methodological structure of the updated OECD guideline 404 (2015) differs in part significantly from the version used and was not yet in place at the time of study conduction. Due to these differences, a comparison of the study with the current guideline would inevitably lead to the identification of number of inherent deviations, which would thus be of limited informative value and could give a distorted picture of its reliability. However, the study reveals some considerable deviations also from the former guideline in terms of methodological and reporting deficiencies. Yet, these were not deemed to have an influence on its overall scientific reliability. Therefore, the study is overall considered reliable with restrictions (reliability score: 2) and being a vertebrate test it is relied upon also for reasons of animal welfare.

In the 1989 study, the skin irritating potential TTO was determined in six young and mature New Zealand White albino rabbits. The test substance was administered undiluted to the intact and abraded skin of mature New Zealand rabbits. All animals were observed for signs of toxicity and abnormal behavior during the experimental period of 72 h. The skin reactions were assessed according to the scoring scheme of Draize.

Overall mean irritation scores of 3.08 and 1.83 for erythema and oedema for intact skin and mean irritation scores of 3.25 and 2.0 for erythema and oedema for abraded skin, respectively were assessed.

10.4.2 Comparison with the CLP criteria

Based on results of study 2 (Anonymous 1989c):means scores for erythema in all tested animals from gradings at 24 - 72 hours after patch removal) were of ≥ 2,3 and ≤ 4,0, therefore Tea Tree Oil has to be classified as skin irritant Category 2 in accordance with the Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures (including all amendments),.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Harmonised classification proposed. Tea Tree Oil has to be classified as Skin irritation category 2 with hazard statement **H315- causes skin irritation**.

10.5 Serious eye damage/eye irritation

The potential of TTO to induce serious eye damage/eye irritation was investigated in rabbits.

Table 23: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Relia-bility score	Reference
Tea Tree Oil: Acute eye irritation/ corrosion study in New Zealand white rabbit OECD 405 (2012) GLP	New Zealand White rabbits Three males	Tea Tree Oil 9.7 % α-Terpinene, 2.6 % 1,8-Cineole, 17.8 % γ-Terpinene, 1.5 % p-Cymene and 41.5 % Terpinen-4-ol (complies with ISO-specification)	1 ml of undiluted test item One administration, observation duration 7 days	Mean total scores for each individual animal 24, 48 and 72 h are presented in Table 24 Reversibility: Conjunctivitis completely regressed on day 7. There were no clinical signs of toxicity. No mortality and abnormal behaviour were observed at necropsy in any of the tested animals.	1	Anonymous 2015d
OECD Guideline 405 GLP	Rabbit (New Zealand White)	Melaleuca alternifolia, ext., Purity 100% (no vehicle)	0.1 mL Tea Tree Oil, purity: 100%	cornea opacity score (animal #1 - mean) 0 of max. 0 (Time point: 24/48/72 h) iris score (animal #1 - mean) 0 of max. 0 (Time point: 24/48/72 h) conjunctivae score – redness (animal #1 - mean) 1 of max. 2 (Time point: 24/48/72 h) fully reversible within: 72 h chemosis score (animal #1 - mean) 0.33 of max. 1 (Time point: 24/48/72 h) fully reversible within: 48 h cornea opacity score (animal #2 - mean) 0 of max. 0 (Time point: 24/48/72 h) iris score (animal #2 - mean) 0 of max. 0 (Time point: 24/48/72 h) conjunctivae score – redness (animal #2 - mean) 0.67 of max. 1 (Time point: 24/48/72 h) fully reversible within: 72 h chemosis score (animal #2 - mean) 0.33 of max. 1 (Time point: 24/48/72 h) fully reversible within: 48 h	1	ECHA dissemination site[23]
In vitro/ex vivo study	Cattle (strain	Melaleuca alternifolia,	0.75 mL of Tea Tree Oil	in vitro irritation score Tea Tree Oil; value 2.2	1	ECHA dissemination

[23] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/4/3

According to OECD Guideline 437 (Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants) [before 26 July 2013] GLP	not specified) 3 corneas for the test material and each control.	ext., Purity 100% (no vehicle)	or control item TTO was applied to isolated bovine corneas for 10 minutes. Duration of post- treatment incubation (in vitro): 120 minutes positive control negative control	Negative controls Irritancy score = 2.3 Positive controls Irritancy score = 44.5		site[24]

10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

Study 1, Anonymous 2015d, Tea Tree Oil: Acute eye irritation/corrosion study in New Zealand white rabbit; OECD 405 (2012); GLP

Reliability statement: The study is conducted in accordance with OECD 405. It meets the validity requirements as set out in the test guideline, shows only minor deviations and the test substance reflects the ISO 4730:2004. Hence, the study is considered reliable without restictions (reliability score: 1).

0.1 ml of the undiluted TTO was installed into the conjunctival sac of the left eye of the three New Zealand White rabbits after gently pulling the lower lid away from the eyeball. The right eye remained untreated and served as a control.

The eye of rabbit were examined at 1, 24, 48, 72 hours and on the 7th day post-instillation and scored.

[24] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/4/3/?documentUUID=e5d53c32-8d2a-4375-9a5c-a878b16d59c4

Table 24: Mean eye irritation scores

Rabbit No.	Time after appl.	CONJUNCTIVA			IRIS	CORNEA	
		Redness A	Chemosis B	Discharge C	Pupil D	Opacity E	Area of opacity F
RBa 858	1 hour	0	0	1	0	0	NA
	24 hours	1	1	2	0	0	NA
	48 hours	1	1	1	0	0	NA
	72 hours	1	1	0	0	0	NA
	Day 7	0	0	0	0	0	NA
Mean score 24-72 h		**1.0**	**1.0**	**1.0**	**0.0**	**0.0**	--
Overall mean		1.0			0.0	0.0	
RBa 859	1 hour	1	1	1	0	0	NA
	24 hours	1	1	2	0	0	NA
	48 hours	1	1	1	0	0	NA
	72 hours	1	1	0	0	0	NA
	Day 7	0	0	0	0	0	NA
Mean score 24-72 h		**1.0**	**1.0**	**1.0**	**0.0**	**0.0**	--
Overall mean		**1.0**			**0.0**	**0.0**	
RBa 860	1 hour	1	2	2	0	0	NA
	24 hours	1	1	2	0	0	NA
	48 hours	1	1	1	0	0	NA
	72 hours	1	1	1	0	0	NA
	Day 7	0	0	0	0	0	NA
Mean score 24-72 h		**1.0**	**1.0**	**1.3**	**0.0**	**0.0**	--
Overall mean		1.1			0.0	0.0	

NA – not applicable Total score: sum of conjunctive, iris and cornea

No mortality was observed during the study. The individual mean scores of eye reactions are reported inTable 24.

A study was performed to assess the irritancy potential of Tea Tree Oil to the eye of the New Zealand White rabbit (ECHA dissemination site). A single application of Tea Tree Oil to the non-irrigated eye of two rabbits produced mean conjunctival redness and chemosis scores of < 2 following grading at 24, 48 and 72 hours. The treated eyes of both animals appeared normal at the 72-hour observation.

A study to assess the ocular irritancy potential of Tea Tree Oil to isolated bovine cornea (ECHA dissemination site) concluded that tea tree oil was not an ocular corrosive or severe irritant.

10.5.2 Comparison with the CLP criteria

Based on these results of two *in vivo* studies the mean scores following grading at 24, 48 and 72 hours after installation of the test material did not meet in any tested animals any of the following criteria for category 2 for reversible effects on the eye according to Regulation (EC) No 1272/2008: corneal opacity ≥ 1 and/or iritis ≥ 1, and/or conjunctival redness ≥ 2 and/or conjunctival oedema (chemosis) ≥ 2., Tea Tree Oil does not have to be classified and has no obligatory labelling requirement for eye irritation according to the Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures (including all amendments).

10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Tea Tree Oil does not need to be classified for serious eye damage or eye irritation. Data is conclusive but not sufficient for classification.

10.6 Respiratory sensitisation

No data on respiratory sensitisation available. Tea Tree Oil was negative in two skin sensitisation studies (see below); therefore, it is unlikely that it would induce respiratory sensitisation.

10.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

Not relevant.

10.6.2 Comparison with the CLP criteria

Not relevant.

10.6.3 Conclusion on classification and labelling for respiratory sensitisation

No classification proposed.

10.7 Skin sensitisation

The skin sensitising potential of Tea Tree Oil was investigated in two guinea-pig maximisation tests according to OECD guideline 406 in 2015 and 1989 and four LLNA studies provided in REACH registration dossier (available on ECHA dissemination site).

Table 25: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reliability score	Reference
Tea Tree Oil: Skin Sensitization Study (Magnusson and Kligman) in Guinea Pigs OECD 406 (1992) GLP	Guinea-Pig Albino, NIH (Duncan Hartley) males and females 10 per control, 20 in the test item group	Tea Tree Oil 9.7 % α-Terpinene, 2.6 % 1,8-Cineole, 17.8 % γ-Terpinene, 1.5 % p-Cymene and 41.5 % Terpinen-4-ol	Induction: 25% (w/w) in propylene glycol Boosting: 50% (w/w) in acetone Challenge: 100% TTO (undiluted) Test duration was 48 h	In the control and treatment group, there were no skin reactions at 24 and 48 hours post removal of the test patch. In the positive control group, 6/10 guinea pigs had score of 1 (discrete or patchy erythema) at 24 and 48 hours post removal of the test patch.There were no clinical signs of toxicity. No mortality was observed during the study.	1	Anonymous 2015e
Skin sensitization potential in the guinea-pig of Tea Tree Oil batch 88/375 OECD 406 (Magnusson & Kligmann)	Guinea-Pig HA-strain 20 animals	Tea Tree Oil	Two weeks after induction application, the test group animals were challenged by application	No dermal responses at challenge. No mortality and abnormal behaviour was observed in all the tested animals during	2	Anonymous 1989d

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reliability score	Reference
GLP not stated			of the maximum sub irritant concentration of the test compound (30% (w/w) dilution of TTO in petroleum jelly) on one flank under occlusive conditions for a period of 24 hours.	the test period.		
Skin sensitisation: *in vivo* (LLNA) According to OECD Guideline 429 (Skin Sensitisation: Local Lymph Node Assay); GLP	Mouse (CBA/CaHsdRcc (SPF)) Female 5/dose/group	Melaleuca alternifolia, ext., Purity 100% (ISO 4730) Stable under storage conditions.	2%, 20% PEG 300 and 100% a negative control group was treated with PEG 300 used as vehicle. Positive control: alpha-hexylcinnam-aldehyde in acetone/ olive oil (4/1 v/v)	Stimulation index (SI) (Mean): 2.4 at 2% (SD=1.4) SI (Mean): 6.9 at 20% (SD=2.0) SI (Mean): 16 at 100% (SD=6.3) EC3=4.4%(w/v) Positive control results provided in study 2006a, below	1	ECHA dissemianation site (study report 2006)[25]
Skin sensitisation: *in vivo* (LLNA) According to OECD Guideline 429 (Skin Sensitisation: Local Lymph Node Assay); GLP	Mouse (CBA/CaHsdRcc (SPF)) Female 5/dose/group	Melaleuca alternifolia, ext., Purity 100% (ISO 4730) Stable under storage conditions.	2%, 20% PEG 300 and 100% a negative control group was treated with PEG 300 used as vehicle. Positive control: alpha-hexylcinnam-aldehyde in	SI (Mean): 1.6 at 2% (SD=0.4) SI (Mean): 2.8 at 20% (SD=0.7) SI (Mean): 5.7 at 100% (SD=1.6) EC3=25.5% (w/v) Positive control results:	1	ECHA dissemination site (study report 2006a)[26]

25 https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/5/2/?documentUUID=d97775d8-17e8-47b6-82c5-bad02fd3e225

26 https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/5/2/?documentUUID=476b7293-ced5-48a6-b1d3-c51085f726b8

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reliability score	Reference
			acetone/ olive oil (4/1 v/v)	SI (Mean): 1.8 at 5% SI (Mean): 2.9 at 10% SI (Mean): 6.2 at 25% EC3=10.5% (w/v)		
Skin sensitisation: *in vivo* (LLNA) According to OECD Guideline 429 (Skin Sensitisation: Local Lymph Node Assay); GLP	Mouse (CBA/CaHsdRcc (SPF)) Female 5/dose/group	Melaleuca alternifolia, ext., Purity 100% (ISO 4730) Stable under storage conditions	2%, 20% PEG 300 and 100% a negative control group was treated with PEG 300 used as vehicle. Positive control: alpha-hexylcinnam-aldehyde in acetone/ olive oil (4/1 v/v)	SI (Mean): 1.8 at 2% (SD=0.4) SI (Mean): 2.8 at 20% (SD=1.2) SI (Mean): 6.5 at 100% (SD=2.3) EC3=24.3% (w/v) Positive control results provided in study 2006a, above	1	ECHA dissemination site (study report 2006b)[27]
Skin sensitisation: *in vivo* (LLNA) No guideline followed, method similar to OECD Guideline 429 GLP	Mouse (CBA/J) Female 5/dose/group	Melaleuca alternifolia, ext., Purity 100% (ISO 4730) Stable under storage conditions, according to Test Article Characteriza-tion	5%, 25% and 50% in PEG 400 a negative control group was treated with PEG 400 used as vehicle. Positive control: alpha-hexylcinnam-aldehyde 25% in PEG 400	SI (Mean): 2.1 at 5% (SD=0.7) SI (Mean): 7.7 at 25% (SD=4.0) SI (Mean): 7.9 at 50% (SD=3.2) EC3=8.3% (w/v) Positive control results: SI (Mean): 21.2 at 25% (SD=7.7)	2	ECHA dissemination site (study report 2007)[28]

Literature studies

[27] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/5/2/?documentUUID=113e4b90-df3c-407d-8c4d-d213eeac7dcb

[28] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/5/2/?documentUUID=2ea26c41-7e89-421f-9540-4bdf0ab30d7b

Open scientific literature search has been performed and some studies on skin sensitisation of the Tea Tree Oil components have been found.

Reliability statement: The literature studies from which the data listed in the table below are derived have not been performed according to official test guidelines. Therefore, it is not possible to evaluate their compliance by identifying any deviations or comparing them against guideline-specific quality criteria. Nonetheless, the publications in question have been assessed for relevance and scientific reliability according to the criteria set out in the EFSA guidance for submission of scientific literature (EFSA Journal 2011;9(2):2092) and assigned a respective reliability score. Details of this process are described in the literature section (CA 9) of the active substance dossier, which clarifies that all studies used in the dossier were classified by the applicant as reliable (reliability score: 1) or reliable with restrictions (reliability score: 2, supporting information).

Table 26: Summary table of other studies relevant for skin sensitisation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reliability score	Reference
Contact allergy to essential oils cannot always be predicted from allergy to fragrance markers in the baseline series. Reliable with restrictions Supportive information • No OECD guideline or GLP defined • Non-validated test system	*Melaleuca alternifolia* oil (composition not specified)	Test system: patch test on human skin Concentrations tested: 5% in petrolatum Number of test individuals: 2104 patients Readings mostly on day 2 and 4, but in some patients on day3 and 5.	Reactions after: 11 (0.5%) positive, 2 (0.1%) doubtful, 3 (0.1%) irritant	2	Sabroe, R.A.. Holden C.R., Gawkrodger, D.J. (2016) Contact Dermatitis, 74, 236-2111
Is tea trea oil an important contact allergen? Reliable with restrictions Supportive information • No OECD guideline or GLP defined • Non-validated test system	*Melaleuca alternifolia* oil (meeting Australian standard for min. + max concentrations of e.g. 1.8-Cineol, d-Limonene, Aromadendrene, α-Terpinene, Terpinolene and α-Pinene)	Test system: Patch test on human skin Concentrations: 10 % in pet., 5 % commercial lotion Number of test individuals: 217 patients (140 ♀, 77 ♂) Placing of patch with test substance on upper back, removed after 2 days, assessment after 3 days	50 of 140 women and 15 of 77 men had 1 or more positive patch test. 1♀: ++ to 10% TTO in pet. and lotion (5% TTO) 3x (1.4%) non-relevant weakly positive reaction to the lotion containing 5% TTO 44 patients (20.3%) had weak, irritant reaction to the lotion	2	Veien, N.K., Rosner, K., Skovgaard, G.L. (2004) Contact Dermatitis 50(6):378-9
		Test system: Patch test on human skin	No allergic reactions 5 patients (3.1%)		

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Relia-bility score	Reference
		Concentrations:4commercial lotions containing 5 % TTO Number of test individuals: 160 patients (117 ♀, 43 ♂) Placing of patch with test substance on upper back, removed after 2 days, assessment after 3 days	irritant reactions		
Comparison of human skin irritation patch test data with in vitro skin irritation assays and animal data. Irritation measurement: 4-hr HPT (human patch test) Reliable with restrictions Supportive information • No OECD guideline or GLP defined • Non-validated test system	α-terpineol (purity 95%)	Test system: 4-hr HPT (human patch test) Concentrations: 0.2 mL, undiluted Number of test individuals: 30 human volunteers Placing of patch with test substance on upper outer arm. 4 hours exposure time, Observations after 24. 48, 72 hours after patch removal	4 hour HPT: Non irritating Positive reactions: 0/29 Positive reactions to SLS: 23/29 (pos. control)	2	Jírová, Basketter, D., Liebsch, M., Bendová, H., Kejlova, K., Marriott, M., Kándarová, H.; 2010 Contact Dermatitis 2010 (62): 109-116
α-Terpinene, an antioxidant in Tea Tree Oil, autoxidizes rapidly to skin allergens on air exposure. Sensitization measurement: Following OECD 429 Reliable with restrictions Supportive information No GLP	α-Terpinene, p-Cymene	Test system: Female CBA/Ca mice Concentrations tested: 0.1, 1, 5, 10 and 30% w/v No. of animals per treatment group: 3 The sensitizing potential of p-Cymene, a degradation product of α-Terpinene, was investigated with the murine local lymph node assay. 8 week old female CBA/Ca mice were used, with test concentration of 0.1, 1, 5, 10 and 30%. 5 h after exposure, the draining auricular lymph nodes were excised and the relative [^{3}H]thymidine incorporation was measured by β-scintillation. Results were expressed as mean dpm/lymph node for each	At any dose tested, p-Cymene did not reach SI values above 3. The EC_3 value was determined as >30% and therefore considered as weak sensitizer in the LLNA assay. For α-Terpinene, EC3 values of 0.9 and 1.0 were determined. It is therefore considered as strong sensitizer.	2	Rudbäck, J., Bergström, M.A., Börje, A., Nilsson, U. (2012) Chemical Research in Toxicology 25: 713 - 721

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Relia-bility score	Reference
		experimental group and as stimulation index (SI) i.e., test group/control group. Test material that caused an SI greater than 3 were considered to be positive in the LLNA. EC3 values were calculated by linear interpolations. Sensitization potency of the test compound was classified according the following: <0.1 extreme; ≥ 0.1 - <1 strong; ≥ 1 - < 10 moderate; ≥ 10 - <100 weak.			
Assessment of sensitization potential of monoterpenes using the rat popliteal lymph node assay. Reliable with restrictions Supportive information • No OECD guideline or GLP defined • Non-validated test system	(-)-menthol 1,8-cineole (+/-) citronellal (+)-limonene (+/-) camphor terpineol.	Test system: Female Wistar rats Test concentrations: Primary assay: 0.5, 2.5 or 5 mg; secondary assay: 0.5 mg The rat popliteal lymph node assay (PLNA) has been used to evaluate the immuno-sensitizing potential of 10 monoterpenes. The primary or direct PLNA was performed with the monoterpenes, and chlorpromazine (CPZ) and barbital were used as positive and negative controls, respectively. Female, 7-8 week-old Wistar rats were injected subcutaneously (50 µL) with the test substance (0.5, 2.5 or 5 mg) into the right hind footpad while the contralateral footpad was injected with the vehicle (DMSO) alone. Weight (WI) and cellularity (CI) indices for draining PLNs were determined 7 days after treatment. A secondary PLNA, a T-cell priming test, was carried out with the four substances that had been positive in the primary assay. Six weeks after being locally primed with 5 mg/paw, rats were sc injected into the same footpad with a dose (0.5 mg/paw) of the substance that had been previously found to be insufficient to cause a positive response. WI and CI were	Primary assay: PLNA was positive (WI >or= 2 and CI >or= 5) for CPZ, citral, alpha-terpinene, beta-myrcene and (-)-alpha-pinene, and negative for barbital, DMSO, (-)-menthol, 1,8-cineole, (+/-) citronellal, (+)-limonene, (+/-) camphor and terpineol. Secondary assay: CPZ was also positive in the secondary assay thereby confirming that it is a sensitizing agent. Citral, alpha-terpinene, beta-myrcene and (-)-alpha-pinene, however, were negative in the secondary assay. In summary, no monoterpene proved to be a sensitizing agent in the PLNA.	2	Friedrich, K., Delgado, I., Santos, L., Paumgartten, F. (2007) Food and Chemical Toxicology

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Relia-bility score	Reference
		then calculated 4 and 7 days after the second injection. No. of animals per treatment group: (-)-menthol 9 1,8-cineole 10 (±)-citronellal 10 (+)-limonene 10 (±)-camphor 10 Terpineol 10 Barbital 8 DMSO 47 Saline 50 Citral 7, 8, 10* α-Terpinene 7, 7, 10* β-Myrcene 7, 8 ,11* (-)-α-pinene 8, 8, 10* Chlorpromazine 4, 6, 11* *: Dose 0.5, 2.5, 5.0 mg/paw			
Limonene hydroperoxide analogues differ in allergenic activity. Reliable with restrictions Supportive information • No OECD guideline or GLP defined	R-limonene Oxidized limonene	Test system: Mice Test concentrations: R-limonene: 25, 50 and 100% w/v , oxidized limonene: 1, 5 and 25% w/v No. of animals per treatment group: 4 The sensitizing potential of R-Limonene was investigated with the murine local lymph node assay. 4 mice per treatment group were used, with test concentration of 25, 50 and 100%. The draining auricular lymph nodes were excised and the relative [^{3}H]thymidine incorporation was measured by β-scintillation. Results were expressed as mean dpm/lymph node for each experimental group and as stimulation index (SI) i.e., test group/control group. Test material that caused an SI greater than 3 were considered to be positive in the LLNA. EC3 values were calculated by linear interpolations. Sensitization potency of the test compound was classified according the following: <0.1 extreme; ≥ 0.1 - <1 strong; ≥ 1 - < 10 moderate; ≥ 10 - <100 weak.	At 50 and 100 %, R-Limonene showed SI values above 3, thus had to be considered as positive in the LLNA The EC3 value was determined to be 30% and therefore considered as weak sensitizer in the LLNA assay.	2	Christensson, J.B., Johansson, S., Hagvall, L., Jonsson, C., Börje, A., Karlberg, A.T. (2008) Contact Dermatitits 59(6): 344-352

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

The skin sensitising potential of Tea Tree Oil was investigated in two guinea-pig maximisation tests according to OECD guideline 406 in 2015 and 1989 and four LLNA studies provided in REACH registration dossier (available on ECHA dissemination site).

Furthermore, six literature studies with different components of Tea Tree Oil are summarized above, three of these studies reported effects on human skin. A further publication reporting effects on human skin is presented below.

Study 1, Anonymous 2015e, Tea Tree Oil: Skin Sensitization Study (Magnusson and Kligman) in Guinea Pigs; OECD 406 (2012); GLP

Reliability statement: The study is conducted in accordance with OECD 425. It meets the validity requirements as set out in the test guideline, shows only minor deviations and the test substance reflects the ISO 4730:2004. Hence, the study is considered reliable without restictions (reliability score: 1).

In the new OECD study, in the control group, at 24 and 48 hours (post administration) observation period erythema score of 1 and oedema score of 1 was observed in 10/10, 0/10 and 10/10 at sites 1, 2 and 3, respectively.

In the treatment group, at 24 hours (post administration) observation period erythema score of 1 was observed in 20/20, 20/20 and 20/20 at sites 1, 2 and 3, respectively. Oedema score of 1 was observed in 20/20, 16/20 and 17/20 at sites 1, 2 and 3, respectively.

At 48 hours (post administration) observation period erythema score of 1 was observed in 20/20, 20/20 and 20/20 at sites 1, 2 and 3, respectively. Oedema score of 1 was observed in 20/20, 13/20 and 14/20 at sites 1, 2 and 3, respectively. There were no clinical signs of toxicity and pre-terminal deaths were observed.

Study 2, Anonymous 1989d, Skin sensitization potential in the guinea-pig of Tea Tree Oil batch 88/375 OECD 406.

Reliability statement: The study was accepted during previous evaluation and appears to be conducted in overall accordance with OECD 406. Apart from that, it shows some deviations in terms of methodology and shortcomoings in reporting, which were, however, not deemed to have an influence on its overall scientific reliability. The study is therefore considered reliable with restictions (reliability score: 2).

In the old OECD study, following administration of the topical challenge dose, animals were free of irritation responses at challenge. On the basis of the results obtained in the GPMT test performed according to the method of Magnusson and Kligmann, the tested TTO did not demonstrated a skin sensitizing potential.

A GLP-compliant LLNA study (ECHA dissemination site, study report 2006) was carried out to determine the possible contact allergenic potential of Tea Tree Oil. The study followed the requirements of EU method B.42, OECD method 429, without significant deviation. Three groups each of five female mice were treated with the test item at concentrations of 2%, 20% (w/v) in PEG 300 and 100% (undiluted) by topical application to the dorsum of each ear lobe (left and right) on three consecutive days. A negative control group of five mice was treated with an equivalent volume of the vehicle polyethylene glycol 300 (PEG 300) only. Five days after the first topical application the mice were injected intravenously into a tail vein with radio-labelled thymidine (^{3}H-methyl thymidine, ^{3}HTdR). Approximately five hours after intravenous injection, the mice were sacrificed, the draining auricular lymph nodes excised and pooled per animal. Single cell suspensions of lymph node cells were prepared from pooled lymph nodes which were washed subsequently and incubated with trichloroacetic acid overnight. The proliferative capacity of pooled lymph node cells was determined by the incorporation of ^{3}HTdR measured in a β-scintillation counter. All treated animals survived the scheduled study period.

Neither clinical/local signs nor other findings were observed in any animals of the control group. One day after the first or the second topical application, a slight ear erythema was observed at both dosing sites in all mice of Group 2 (2%), Group 3 (20%) and Group 4 (100%, undiluted), persisting for the remainder of the in-life phase of the study (Groups 3-4), or persisting for a total of two days (Group 2). In addition, two days after the

third topical application (Day 5) and prior to sacrifice (Day 6), scales were found on both ears in all mice of Group 4 (100%, undiluted). No significant difference of dpm/ LN was determined at the test item concentration of 2% (w/v) in PEG 300 compared with the vehicle control group at $p \leq 0.05$ (two sides). A significant difference of dpm/LN was determined at the test item concentrations of 20% in PEG 300 and 100% (undiluted) compared with the vehicle control group at $p \leq 0.05$ (two sides).

A test item is regarded as a sensitizer in the LLNA if the exposure to one or more test concentrations resulted in 3-fold or greater increase in incorporation of ^{3}HTdR compared with concurrent controls, as indicated by the S.I. In this study S.I. of 2.4, 6.9 and 16.0 were determined with the test item at concentrations of 2%, 20% (w/v) in PEG 300 and 100% (undiluted), respectively. Tea Tree Oil was therefore found to be a potential skin sensitizer and an EC3 value of 4.4% (w/v) was derived.

The second LLNA study (ECHA dissemination site, study report 2006a) was conducted in the same way as above study however no erythema or scales were found on ears of all mice after topical application. In this study S.I. of 1.6, 2.8 and 5.7 were determined with the test item at concentrations of 2 %, 20 % (w/v) in PEG 300 and 100 % (undiluted), respectively. Tea Tree Oil was therefore found to be a potential skin sensitizer and an EC3 value of 25.5 % (w/v) was derived.

The third LLNA study (ECHA dissemination site) was conducted in the same way as above two studies. One day after the second topical application (Day 3) and on Day 4, a slight ear erythema and hypersensitivity to touch on both ears were observed at both dosing sites in all mice of Group 2 (2%). One day after the first topical application (Day 2), a slight ear erythema was observed at both dosing sites in all mice of Group 3 (20%), persisting for the remainder of the in-life phase of the study. On Days 3-4, the ears of all mice in this group were hypersensitive to touch (both ears). On Day 6 (prior to necropsy), scales were found on both ears in all mice of Group 3 (20%). One day after the first or the second topical application, a slight to moderate ear erythema and/or slight ear swelling were observed at both dosing sites in all mice of Group 4 (100%, undiluted), persisting for the remainder of the in-life phase of the study. On Days 3-4, the ears of all mice in this group were hypersensitive to touch. On Day 4, scales were found on both ears in all mice of this group, persisting for the remainder of the in-life phase of the study. In this study S.I. of 1.8, 2.8 and 6.5 were determined with the test item at concentrations of 2%, 20% (w/v) in PEG 300 and 100% (undiluted), respectively. Tea Tree Oil was therefore found to be a potential skin sensitizer and an EC3 value of 24.3% (w/v) was derived.

The GLP-compliant study (ECHA dissemination site, study report 2007) followed MB Research Protocol 5650A-06 and determined the sensitizing potential of topically applied Melaleuca alternifolia. A preliminary screening test was conducted with three groups of healthy female CBA/J mice (2 per group) to determine the concentrations of the test article to be used in the main study. Because the chosen vehicle, PEG 400, had not been validated, two additional groups of 2 mice each were added, one treated with the PEG 400 vehicle and one treated with 25% HCA in PEG 400. The initial screening study showed no irritation at any of the test article concentrations tested, including the maximum soluble concentration of 50%. No irritation was detected following treatment of the PEG 400 vehicle, whereas 25% HCA in PEG 400 elicited irritation. Concentrations of 5%, 25% and 50% of the test article were chosen for the definitive Local Lymph Node study by the sponsor.

For the definitive study, three separate groups of five healthy female CBA/J mice were treated with increasing concentrations of Tea Tree Oil by topical application to the dorsum of each ear, once daily for three consecutive days. A vehicle control group of five mice was treated with PEG 400 and another group of five mice were treated with the positive control, 25% HCA (in PEG 400), in exactly the same manner.

Five days following the initial dose, and five hours prior to sacrifice, the mice were given an intraperitoneal injection of the thymidine analog 5-bromo-2'deoxy-uridine (BrdU), and at sacrifice the auricular lymph nodes were isolated and single-cell suspensions of lymph node cells (LNC) were generated. For each animal, the LNC suspension was analysed for BrdU incorporation and total number of LNC by flow cytometry. The amount of proliferating (BrdU+) LNC was determined as a measure of the proliferative response of the local lymph node. The stimulation index (SI) was calculated by dividing the proliferative response (BrdU incorporation) of each test article group by the proliferative response of the vehicle control group. Test articles that yielded a $SI \geq 3$ were characterized as sensitizing substances.

All animals survived the in-life phase of the study and appeared normal. Body weight changes were normal. Ear swelling measurements and individual animal observations indicated that none of the treatments resulted in dermal irritation.

The SI of the positive control, 25% HCA, was 21.2, similar to 25% HCA in more common vehicles. The SI values for the test article at 5%, 25% and 50% were 2.1, 7.7 and 7.9, respectively. Since topical application of the test article at 25% and 50% in PEG 400 resulted in a stimulation index greater than 3, this test article is considered to be a dermal sensitizer in the Local Lymph Node Assay. The EC3 for this test article was calculated to be 8.3%, which would classify it as extremely weak for sensitizing potency.

Publication (Review): Larsen, J.R. and Borling, P. (2000), Tea Tree Oil. Safety aspects. Danish Toxicology center (DTC), Denmark. Publication evaluated under Reg. 91/414 and presented in the Draft Assessment Report of TTO (2007), Vol. 3, Annex B.6.

This review of the toxicity of TTO contains references to 35 peer reviewed scientifc pubilications. Those addressing effects on skin after topical application are reported in the following.

28 volunteers were treated with 25% TTO, containing 1.5-28.8 of 1,8-Cineole. Irritancy was not detected, however, 3 of the 28 had severe allergic response.

Seven patients with pre-existing skin conditions were patch-tested with 11 constituents of TTO.

All seven patients were reactive to 1% TTO. In addition, six reacted to Limonene, five to α-terpinene and aromadendrene, two to terpinen-4-ol and one each to p-cymene and α-phellandrene.

In Denmark since 1991 more than 30 cases of patients sensitized by using TTO topically have been documented. About 5 new cases of allergy to TTO out of 1000 patients are seen per year.

Oxidised TTO caused three times stronger reaction than freshly distilled solutions, monoterpene fraction was stronger sensitizer than the sesquiterpene fraction and the sensitizing constituents were p-cymene, aromadendrene, ascaridol, terpinolene and α-terpinene. TTO undergoes photooxidation within a few days to several months, leading to creation of sensitizing degradation products (*e.g*. ascaridol).

A 45-year-old man with a long history of dermatitis was dermally treated with undiluted TTO and experienced worsening of his dermatitis after an initial improvement. He was then advised to ingest the oil mixed with honey (dose unknown), which resulted in obvious exacerbation of the dermatitis. A patch test with the main ingredients of Tea Tree Oil revealed 1,8-cineole to be the actual allergen.

A 33-year-old woman, who had been treating her acne for several years with Tea Tree Oil, was presented with a 1-week history of dermatitis. Patch test showed a reaction at the site of Tea Tree Oil and at the site of colophony. Cross reaction between colophony and oil of turpentine has previously been reported. The actual allergen in Tea Tree Oil proved to be 1,8-cineole.

A 74-year-old man developed contact dermatitis from Tea Tree Oil in wart paint within 24 hours of application. Patch test showed a reaction towards Tea Tree Oil (1 %) and also to fragrance mix, but no reactions were seen to the individual constituents of Tea Tree Oil that were tested.

The RMS concluded that this publication can be relied upon and that the effect of TTO is related to skin irritation and sensitization in humans after dermal contact. The symptoms initially observed were reversible and recovery was demonstrated. Fresh TTO seems to be better tolerated so a date of minimum durability should be considered.

Further literature studies of single TTO components were provided as supportive data. It was described that in LLNA (or PLNA) the tested terpenes can be considered as weak or non-sensitizing.

In the following table, the test results are compared to the results in the ECHA C&L database.

Compound	**Test results**	**ECHA C&L database**
α-terpineol (purity 95%)	not irriating not sensitizing (PLNA)	Skin irrit. 2
α-Terpinene CAS no.: 99-86-5	pure: moderate sensitizer, strong sensitizer after bioactivation and	RAC Opinion no CLH-O-0000001412-86-274/F:

(content in TTO 5-13%)	autoxidation	Skin Sens. 1
p-Cymene	weak sensitizer	Skin irrit. 2
1,8-cineole	not sensitizing (PLNA)	Skin-sens. 1B
Limonene CAS no.: 138-86-3 (content in TTO 0.5-1.5%)		Harmonised classification - Annex VI of CLP Regulation: Skin Sens. 1

10.7.2 Comparison with the CLP criteria

In accordance with the Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures (including all amendments), a sensitising potential of a substance is identified, if response in a guinea-pig maximisation tests at least 30% of the animals is considered as positive (redness score ≥ 1).

Within the available GLP compliant GPM test, no erythema was observed in any of the test animals after intradermal induction with a test item concentration of 25 % (w/v). In a second GPMT which was not reported in such detail, but which was also performed according to the method by Magnusson & Kligmann, there were again no animals with signs of erythema.

Hence, since no positive reactions were observed in GPMT for >30% of treated animals the criteria for classification with regard to skin sensitization are not met for Tea Tree Oil.

It is noted that four LLNA are available for TTO in REACH registration dossier. TTO concentrations tested in LLNAs were between 2-100%. The stimulation index obtained in the LLNAs at concentration of 2% was between 1.6 – 2.4. The EC3 values for TTO tested in LLNAs at concentrations above 2% and up to 100% ranged between 4.4% and 25.5% thus TTO meets classification criterion as a skin sensitiser. Noting that subcategory 1A can be excluded since at concentrations of 2% TTO did not induced stimulation index above 3, the substance warrants classification Skin Sens.1B because the EC3 values found in several LLNAs were above 2%.

It is also noted that α-Terpinene present in TTO at concentrations 5-13% has been classified as Skin Sens.1 in the RAC Opinion no CLH-O-0000001412-86-274/F and and Limonene present in TTO at concentrations of 0.5-1.5% has harmoinised classification as Skin Sens.1 (Annex VI of CLP Regulation)

10.7.3 Conclusion on classification and labelling for skin sensitisation

Based on all provided data, classification as Skin Sens. 1B, with hazard statement H317: May cause an allergic skin reaction is proposed for Tea Tree Oil

10.8 Germ cell mutagenicity

The genotoxic properties of Tea Tree Oil were investigated with *in vitro* tests (bacterial reverse mutation test, mammalian cell gene mutation test, mammalian micronucleus test, mammalian chromosomal aberration test) and with an *in vivo* test for DNA damage (mouse micronucleus test).

Table 27: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reliability score	Reference
Bacterial reverse mutation test OECD 471 (1997) GLP	Tea Tree Oil 9.13% α-Terpinene, 1.55 % 1,8-Cineole, 18.45% γ-Terpinene, 1.66 % p-cymene and 40.50% Terpinen-4-ol (analyzed)	Test system: TA 98, TA 100, TA 1535 and TA 1537 strains of *Salmonella typhimurium* and WP2 uvrA (pKM 101) strain of *Escherichia coli*. Concentrations tested: Initial test: 50, 158, 500, 1581 and 5000 µg/plate (± S9) Confirmation assay: 100, 266, 707, 1880 and 5000 µg/plate (± S9) (Concentration selection based on a cytotoxicity pre-tests)	Tea Tree Oil was not mutagenic in this bacterial reverse mutation assay up to the highest tested concentration of 5000 µg/plate.	1	Anonymous (2010b)
In vitro mammalian cell gene mutation test OECD 476 (1997) GLP	Melaleuca alternifolia, ext.	mouse lymphoma L5178Y cells Assay 1: - With S9-mix (3 h treatment): Cells evaluated at 100, 75, 50, 25, 10 and 5 µg/mL. - Without S9 mix (3 h treatment): Cells evaluated at 70, 60, 40, 20, 10 and 5 µg/mL Positive control substance(s): 4-nitroquinoline-N-oxide; cyclophosphamide Assay 2: - With S9-mix (3 h treatment): Cells evaluated at 125, 112.5, 100, 75, 50, 25, 10 and 5 µg/mL - Without S9 mix (24 h treatment): Cells evaluated at 40, 30, 20, 10 and 5 µg/mL. Positive control substance(s): 4-nitroquinoline-N-oxide; cyclophosphamide	No mutagenic effect of tea tree oil nor any formed metabolites was observed either in the presence or absence of a metabolic activation system under the conditions of this Mouse Lymphoma Assay.	1	ECHA dissemination site(study report 2010)[29]
In Vitro Mammalian Chromosome Aberration Test OECD 473	Melaleuca alternifolia, ext.	Chinese hamster lung fibroblasts (V79) [mammalian cell line] (Met. act.: with and without) Experiment A with 3/20h treatment/ sampling time:	Tea Tree Oil tested up to cytotoxic concentrations, both with and without metabolic activation, did not induce structural	1	ECHA dissemination site (study report 2009)[30]

[29]https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/7/2/?documentUUID=78a01fb0-6711-46b3-8e26-748d62eba1dc

[30] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/7/2

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reliability score	Reference
(2016) GLP		- Without S9 mix: 3.12 µl DMSO/mL (solvent control); 9.76, 19.53, 39.06 and 58.59 µg Tea Tree Oil/mL (metaphase analysis conducted at these concentrations). A treatment at 78.12 µg TTO/mL was not assessed because of very low survival. Positive control (Ethyl methanesulphonate): 1.0 µl/mL. - With S9 mix (50 µl/mL): 3.12 µl DMSO/mL (solvent control); 9.76, 19.53, 39.06 and 58.59 µg Tea Tree Oil/mL (metaphase analysis conducted at these concentrations). A treatment at 78.12 µg TTO/mL was not assessed because of very low survival. Positive control (N-Nitrosodimethylamine): 1.0 µl/mL. Experiment B with 20/28h treatment/ sampling time: - Without S9 mix: 2.34 µl DMSO/mL (solvent control); 4.88, 9.76, 19.53 and 39.06 µg Tea Tree Oil/mL (metaphase analysis conducted at these concentrations). Metaphase analysis was not conducted in a treatment at 58.59 µg Tea Tree Oil/mL. Positive control (Ethyl methanesulphonate): 0.4 µl/mL. Experiment B with 3/28h treatment/ sampling time: - With S9 mix (50 µl/mL): 3.12 µl DMSO/mL (solvent control); 9.76, 19.53, 39.06 and 58.59 µg Tea Tree Oil/mL (metaphase analysis conducted at these concentrations). A treatment at 78.12 µg TTO/mL was not assessed because of very low survival. Positive control (N-Nitrosodimethylamine): 1.0 µl/mL. Positive control substance(s): ethylmethanesulphonate	chromosome aberrations in this test in V79 Chinese Hamster lung cells. Therefore, Tea Tree Oil and its metabolite(s) are not considered to be clastogenic in this test system.		
Bacterial Reverse Mutation Test	Tea Tree Oil	Test system: TA98, TA100 and TA102 strains of *Salmonella typhimurium*. Concentrations tested:	Tea Tree Oil was negative with regard to the mutagenicity in the presence or absence of metabolic activation in	2	ECHA dissemination site (1989)[31]

[31]https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/7/2/?documentUUID=1ea9fce2-9169-4571-b7f3-8dbad1740e15

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reliability score	Reference
comparable to guideline study with acceptable restrictions GLP no		10, 25, 50, 100, 150 µl µg/plate (± S9) Confirmation assay: 100, 266, 707, 1880 and 5000 µg/plate (± S9) (Concentration selection based on a cytotoxicity pre-tests)	tests with *Salmonella typhimurium* strains TA98, TA100 and TA102, respectively.		
Tea Tree Oil: *In Vitro* Mammalian Cell Gene Mutation Test in CHO Cells OECD 476 (1997) GLP	Tea Tree Oil 9.7% α-Terpinene, 17.8% γ-Terpinene, 2.6% 1,8-Cineole, 1.5% p-Cymene and 41.5% Terpinen-4-ol	Test system: Chinese hamster Ovary cells (CHO) Initial /confirmatory gene mutation assays Concentrations tested: - S9: 8/7, 19/17, 41/39 and 90 µg/mL + S9: 8/7, 19/17, 41/39 and 90 µg/mL (Concentration selection based on a cytotoxicity pre-tests)	There was no evidence of induction of gene mutations in any of the test item treated cultures either in the presence or absence of metabolic activation.	1	Anonymous (2015f)
In vitro mammalian micronucleus test (Similar to OECD 487) GLP not stated	Tea Tree Oil (from *Melaleuca alternifolia* leaves) Terpinen-4-ol (42.8%), γ-terpinene (20.4%), p-cymene (9.6%), α-terpinene (7.9%), 1,8-cineole (3%), α-terpineol (2,8%) and α-pinene (2.4%)	Test system: human lymphocyte cultures Concentrations tested: 95, 182, 365 µg/mL (Concentration selection based on a cytotoxicity pre-tests, determined by reduction in mitotic index) No information on metabolic activation	None of the tested TTO concentrations caused significant increase in the observed frequencies of micronuclei when compared to those in negative control.	2	Pereira, T.S., (2014)
In vitro mammalian chromosomal aberration test (similar to OECD 473) GLP not stated	Tea Tree Oil (from *Melaleuca alternifolia* leaves) Terpinen-4-ol (42.8%), γ-terpinene (20.4%), p-cymene (9.6%), α-terpinene	Test system: human lymphocyte cultures Concentrations tested: 95, 182, 365 µg/mL (Concentration selection based on a cytotoxicity pre-tests, determined by reduction in mitotic index) No information on metabolic activation	No significant differences regarding the frequency of chromosome aberration were observed compared to those in negative control.	2	Pereira, T.S., (2014)

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reliability score	Reference
	(7.9%), 1,8-cineole (3%), α-terpineol (2,8%) and α-pinene (2.4%)				

Table 28: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reliability score	Reference
In vivo micronucleus test of Australian Tea Tree Oil (*Melaleuca alternifolia*) Protocol based on OECD 474 (1997) GLP	Tea Tree Oil	The mutagenicity of Tea Tree Oil was investigated *in vivo* in somatic cells. The test item TTO was administered orally at 3 dose levels (1000 (10% w/w), 1350 and 1750 mg/kg) to 4 groups of 10 animals (5 males and 5 females). The vehicle (corn oil), as a control is tested parallel (20 animals treated with vehicle at 10.61 mL/kg). The positive control (DMBA) was administered by IP injection at 40 mg/kg (8.8 mg/mL). The animals treated with the test item and the control were sacrificed after 24 and 48 hours. 10 animals treated with the positive control were sacrificed 48 hours after administration.	The results of the *in vivo* mouse bone marrow micronucleus assay indicated that TTO did not increase the number of micro nucleated PCE up to and including cytotoxic doses as shown by a statistically significant depression of the ratio of polychromatic erythrocytes to total erythrocytes. Thus, TTO is not mutagenic *in vivo*. In addition, the results indicated that the highest dose of TTO (1750 mg/kg) was however toxic to the tested animals.	1	Anonymous (2005)

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

The genotoxic potential of Teat Tree Oil has been investigated in several *in vitro* and *in vivo* studies. Following table summarizes the results.

Table 29: Summary of genotoxicity testing of Tea Tree Oil

Parameter	Concentration	Results	Reference
***In vitro* studies**			
Bacterial Reverse Mutation Test	50, 158, 500, 1581 and 5000 µg/plate (+/- S9)	Negative	Anonymous (2010b)
Bacterial Reverse Mutation Test	50 µg/plate (+/- S9)	Negative	ECHA dissemination site
Mammalian Cell Gene Mutation Test	8, 19, 41 and 90 µg/mL (+/- S9)	Negative	Anonymous (2015f)
In vitro mammalian chromosomal aberration test	Exp. 1 (3/20 h treatment): 9.76, 19.53, 39.06 and 58.59 µg /mL (+/- S9) Exp. 2 (20/28 h treatment): 4.88, 9.76, 19.53 and 39.06 µg /mL (- S9) Exp. 3 (3/28 h treatment: 9.76, 19.53, 39.06 and 58.59 µg /mL (+ S9)	Negative	ECHA dissemination site
In vitro mammalian cell gene mutation test	Assay 1: 100, 75, 50, 25, 10 and 5 µg/mL/70, 60, 40, 20, 10 and 5 µg/mL (+ S9/-S9) Assay 2: 125, 112.5, 100, 75, 50, 25, 10 and 5 µg/mL/40, 30, 20, 10 and 5 µg/mL (+ S9/-S9)	Negative	ECHA dissemination site
In vitro mammalian micronucleus test	95, 182, 365 and 548 µg/mL	Negative	Pereira *et al.* (2014)
In vitro mammalian chromosomal aberration test	95, 182, 365 and 548 µg/mL	Negative	Pereira *et al.* (2014)
***In vivo* studies**			
Mouse Micronucleus Test	1000 (10% w/w), 1350 and 1750 mg/kg bw	Negative	Anonymous (2005)

The potential of Tea Tree Oil to induce genotoxicity was investigated *in vitro* and *in vivo*. Bacterial gene mutation was negative under GLP and non-GLP conditions with and without metabolic activation. Tea Tree Oil was also tested negative for mammalian cell gene mutation in a guideline conform study. With respect to clastogenicity, two *in vitro* studies were performed. In both, a mammalian micronucleus test and a mammalian chromosomal aberration test, Tea Tree Oil did not show the potential to induce DNA damage *in vitro*. In order to determine whether Tea Tree Oil is able to induce genotoxicity *in vivo*, a mouse micronucleus assay in bone marrow was performed. The test followed an in-house experimental procedure which was based on the OECD GD 474 under GLP conditions. Bone marrow exposure of the absorbed Tea Tree Oil was proven as seen in the significant depression of the PCE and PCE+NCE ratio indicating bone marrow toxicity in high dose animals 48 h after dosing, and also inferred from systemic toxicity (wobbly gait, laboured breathing, rough coat) and ADME studies. Mice treated with the test item at any dose did not reveal an increase in the incidence of

micronuclei (MPCEs) when compared with the negative vehicle control at 24 and 48 hours sampling times. The positive control DMBA induced statistically significant increase in the frequency of MPCEs compared to the vehicle control, demonstrating the validity of the test method. It can therefore be concluded that Tea Tree Oil is not clastogenic *in vivo*.
All in all, there are no indications, neither *in vitro* nor *in vivo* that Tea Tree Oil induces gene mutations or DNA damage. The substance can therefore be considered as non-genotoxic.

Photomutagenicity testing

According to Regulation (EC) No 1107/2009, a photomutagenicity testing with an active substance is required if the UV/VIS molar extinction coefficient (ε) of the active substance is > 1000 L x mol^{-1} x cm^{-1} at 290-700 nm.

The UV spectra of the marker components of Tea Tree Oil have been measured. None of the Tea Tree Oil components noteworthily absorb at > 290 nm in neutral aqueous media (at pH 6). Accordingly, a photomutagenicity testing with the active substance Tea Tree Oil is not required.

For the evaluation of genetic toxicity of Tea Tree Oil several guideline- and GLP-studies were available - sufficient to evaluate the genotoxic potential of TTO. Therefore, studies found in open literature for the single terpene compound of Tea Tree Oil were only briefly summarized below.

Reliability statement: The literature studies from which the data listed in the table below are derived have not been performed according to official test guidelines. Therefore, it is not possible to evaluate their compliance by identifying any deviations or comparing them against guideline-specific quality criteria. Nonetheless, the publications in question have been assessed for relevance and scientific reliability according to the criteria set out in the EFSA guidance for submission of scientific literature (EFSA Journal 2011;9(2):2092) and assigned a respective reliability score. Details of this process are described in the literature section (CA 9) of the active substance dossier, which clarifies that all studies used in the dossier were classified by the applicant as reliable (reliability score: 1) or reliable with restrictions (reliability score: 2, supporting information).

Table: 30

Test substance	Type	Cell/Species	Concentration	Findings	Reliability	Reference
In vitro						
γ-Terpinene	Single-cell gel electrophoresis (basic alkaline comet assay)	Human lymphocytes	0.00005 mM 0.0005 mM 0.005 mM 0.025 mM 0.05 mM 0.1 mM 0.2 mM 0.5 mM 1 mM	No increase in DNA strand breakage was observed at concentrations below 0.1 mM, but at higher concentration of 0.2 mM significant increase in DNA damage was seen.	2	Aydin, S., Basaran, A. A., Basaran, N. (2005)
γ-Terpinene	Single-cell gel electrophoresis (comet assay)	Human lymphocytes	0.00005 mM 0.0005 mM 0.005 mM 0.025 mM 0.05 mM 0.1 mM 0.2 mM 0.5 mM 1 mM	Concentrations above 0.1 mM significantly induced DNA damage in human lymphocytes, but at the lower concentrations no additional DNA strand breakage has been observed.	2	Aydin, S., Basaran, A. A., Basaran, N. (2005)
α-Terpinene	*Drosophila melanogaster* somatic mutation and recombination test (wing spot test)	*Drosophila melanogaster* strains	2.5 µL/mL 5.0 µL/mL 7.5 µL/mL 10.0 µL/mL	The substance has been found to be free from mutagenic activity.	2	Mdemtzoglou, D., Pavlidou, T., Bazioti, M-G., Koutsonikou, C., Lioulia, E., Akmoutsou, P., Drosopoulou, E., Vokou, D., Mavragani-Tsipidou, P. (2013)

Test substance	Type	Cell/Species	Concentration	Findings	Reliability	Reference
Limonene	Single-cell gel electrophoresis (alkaline comet assay) Micronucleus (MN) assays	Human lymphocytes and V79 cells(Comet assay) V79 cells (MN assay)	2000 µM 4000 µM 6000 µM 8000 µM 10000 µM 12000 µM 14000 µM 16000 µM 18000 µM 20000 µM	Limonene at a concentration below 10000µM has no exerted genotoxic affects in lymphocytes and in V79 cells.	2	Bacanli, M., Basaran, A. A., Basaran, N. (2015)
Limonene	Mutagenicity assay acc. to OECD	*Salmonella typhimurium* TA98 and TA100 on *Escherichia coli* WP2*uvrA* strains	0.04 – 0.15 µmol/plate	The test substance was lacking a mutagenic effect.	2	Di Sotto, A., Durazzi, F., Sarpietro, M. G., Mazzanti, G. (2013)
Limonene	*Drosophila melanogaster* somatic mutation and recombination test (wing spot test) SMART assay	*Drosophila melanogaster* strains	0.011 mM 0.73 mM	The test substance was non-mutagenic at lowest concentration. Nevertheless, Limonene was mutagenic in the SMART assay at the highest concentration.	2	Fernández-Bedmar, Z., Anter, J., Cruz-Ares, S., Muñoz-Serrano, A., Alonso-Moraga, À., Pérez-Guisado, J. (2011)
Limonene	*Drosophila melanogaster* somatic mutation and recombination test (wing spot test) SMART assay	*Drosophila melanogaster* strains	1.5 µL/mL 2.5 µL/mL 5.0 µL/mL	Statistical analysis of the study did not show clearly negative activity in the case of Limonene leading to an inconclusive result at the highest dose. Negative mutagenic activity was proven at the lower doses.	2	Mademtzoglou, D., Akmoutsou, P. Kounatidis, I., Franzios, G., Drosopoulou, E., Vokou, D. Mavragani-Tsipidou, P. (2011)
α-Terpineol	Mutagenicity assay acc. to OECD	*Salmonella typhimurium* TA98 and TA100 on *Escherichia coli* WP2*uvrA* strains	1.1 – 4.3 µmol/plate	The test substance was lacking a mutagenic effect.	1	Di Sotto, A., Durazzi, F., Sarpietro, M. G., Mazzanti, G. (2013)
α-Terpineol	*Drosophila melanogaster* somatic mutation and recombination test (wing spot test)	*Drosophila melanogaster* strains	2.5 µL/mL 5.0 µL/mL 7.5 µL/mL 10.0 µL/mL	The substance has been found to be free from mutagenic activity.	2	Mdemtzoglou, D., Pavlidou, T., Bazioti, M-G., Koutsonikou, C., Lioulia, E., Akmoutsou, P., Drosopoulou, E., Vokou, D., Mavragani-Tsipidou, P. (2013)

Test substance	Type	Cell/Species	Concentration	Findings	Reliability	Reference
1.8-Cineole	Single-cell gel (comet) assay	Mouse lymphoma cells	1.25 µL/mL	1.8-Cineole did not induce DNA strand breaks. It is therefore not likely to increase the level of DNA damage on mammalian cells.	2	Ribeiro, D. A., Marques, M. E. A., Salvadori, D. M. F. (2006)
1.8-Cineole	Single-cell gel (comet) assay	Chinese hamster ovary cells	1.25 µL/mL	1.8-Cineole did not induce DNA breakage at 1.25 µL/mL concentration. The results suggest that 1.8-Cineole may not be a factor that increases the level of DNA lesions in mammalian cells	2	Ribeiro, D. A., Matsumoto, M. A., Marquez, M. E. A., Solvadori, D. M. F. (2007)
1,8-Cineole	Alkaline and neutral comet assay	Human coloretal cancer cell line HCT116 Hamster fibroblast cell lines AA8, RAD51D1, V79-2 and VC8	5 µM 50 µM 200 µM	No increase in DNA strand break formation was observed in response to 1,8-Cineole treatment.	2	Dörsam, B, Wu, C., Efferth, T., Kaina, B, Fahrer, B. (2015)
1,8-Cineole	Alkaline comet assay	Vero cell line obtained from the kidney of a normal adult African green monkey *E. coli* bacterial cells	5 µL/plate 7.5 µL/plate 10 µL/plate 15 µL/plate	The analysis of tail moment indicated no genotoxicity up to 10 µM. However, at higher concentrations the indication of genotoxicity was obtained.	2	Nikolić, B., Mitić-Ćulafić, D., Vuković-Gačić, B., Knežević-Vukčević, J. (2011)
1,8-Cineole	Mutagenicity assay	*E. coli* WP2 strains IC185 *trpE65* and its derivative IC202 *trpE65oxy R/*PKM101	0.05 mg/plate 0.1 mg/plate 0.5 mg/plate 1.0 mg/plate 1.5 mg/plate	1,8-Cineole was not mutagenic in IC185 or IC202 strain. In IC202 strain, but not in IC185 strain.	2	Mitić-Ćulafić, D., Žegura, B., Nikilić, B., Vuković-Gačić, B., Filipič, M. (2009)
	Single-cell gel electrophoresis (comet assay)	Human hepatoma cell line (HepG2) Human B lymphoid NC-NC cells	1 µh/mL	The test substance did not induce DNA damages.		
1,8-Cineole	Mutagenicity assay acc. to OECD	*Salmonella typhimurium* TA98 and TA100 on *Escherichia coli* WP2*uvrA* strains	1.5-6.0 µmol/plate	The test substance was lacking of mutagenic effect.	2	Di Sotto, A., Durazzi, F., Sarpietro, M. G., Mazzanti, G. (2013)
α-Pinene	Alkaline single-cell gel electrophoresis (comet assay)	Human lung epithelial A549 cells	1 mg/m³ 20 mg/m³ 1000 mg/m³ 1800 mg/m³	α-Pinene failed to induce DNA migration.	2	Gminski, R., Tang, T., Mersch-Sundermann, V. (2010)

Test substance	Type	Cell/Species	Concentration	Findings	Reliability	Reference
α-Pinene	*Drosophila melanogaster* somatic mutation and recombination test (wing spot test)	*Drosophila melanogaster* strains	2.5 µL/mL 5.0 µL/mL 7.5 µL/mL 10.0 µL/mL	The substance has been found free from mutagenic activity.	2	Mdemtzoglou, D., Pavlidou, T., Bazioti, M-G., Koutsonikou, C., Lioulia, E., Akmoutsou, P., Drosopoulou, E., Vokou, D., Mavragani-Tsipidou, P. (2013)

10.8.2 Comparison with the CLP criteria

The genotoxic properties of Tea Tree Oil were investigated with *in vitro* tests (bacterial reverse mutation test, mammalian cell gene mutation test, mammalian micronucleus test, mammalian chromosomal aberration test) and with an *in vivo* test for DNA damage (mouse micronucleus test).

Tea Tree Oil was not genotoxic in the tests conducted under the test conditions used.

Overall, the weight of evidence indicates that Tea Tree Oil does not pose an *in vivo* mutagenic or genotoxic concern to humans.

No information is available on the genotoxicity of Tea Tree Oil in humans. Therefore, it clearly does not meet the criteria for classification in category 1A. Since Tea Tree Oil was negative in *in vivo* tests in mammals and there is no information on its mutagenicity in germ cells, classification in category 1B is not appropriate, as well.

Classification for germ cell mutagenicity category 2 is not appropriate as the *in vivo* study and the *in vitro* studies have shown that Teat Tree Oil is negative in all assays.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Tea Tree Oil does not need to be classified for germ cell mutagenicity. Data conclusive but not sufficient for classification.

10.9 Carcinogenicity

There are no carcinogenicity studies with Tea Tree Oil available.

A waiver is provided to show that long-term studies with carcinogenicity testing were not necessary for Tea Tree Oil (see ANNEX 2 to CLH report) and is summarized in the following. Additionally, publicly available carcinogenicity data for single Tea Tree Oil components are presented in support of the assessment.

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

TTO/its components occur naturally and are degraded in edible crops and in the environment.

Consumers are exposed to TTO and its components from natural sources (edible crops and environment) and from their use in health care products and as food flavors. TTO had a long history of safe use in a wide range of cosmetic and human and animal care products (mouthwash, toothpaste, shampoo, deodorants, lotions, and antifungal treatment). TTO (*Melaleuca alternifolia* oil) is listed as an ingredient employed in cosmetic products.

Consumers are not exposed to TTO/its components from crops treated with TTO, due to the lack of residues on these treated crops.

Moreover, TTO/its components would be metabolized and cleared rapidly within 2-3 days if entered human body, as it was shown earlier by animal and human volunteers studies. In addition, TTO components were metabolized to innocuous metabolites which are rapidity cleared from the body.

It is noteworthy that due to the rapid clearance from the body, there is no potential for bioaccumulation and there is no specific target organ in which TTO (or components) is incorporated after absorption.

In case a small fraction of TTO/its components remains in the body, it is unlikely to cause any long-term effects, such as carcinogenicity.

It is further notable that TTO was tested negative in genotoxicity studies. It is therefore unlikely that carcinogenicity appears which is based on genotoxic mechanisms of action.

In contrast, several studies demonstrated that TTO and its main component terpinene-4-ol have anti-carcinogenic activities against various types of cancer, both *in vitro* and *in vivo*.

The following carcinogenicity studies with single TTO components were available:

Terpineol (Study information published on ECHA homepage[32])

- In a carcinogenicity study, female mice were given intraperitoneal injections of alpha-terpineol or beta-terpineol at 1900 and 9600 mg/kg bw in tricaprylin, 3 times a week for a total of 24 doses. Animals were then observed for mortality and bodyweights for 24 weeks after first injection and were all macroscopically necropsied after death or sacrifice. No dose-related increase was observed in tumor formation.
- **Under the test conditions, β terpineol was not considered to be carcinogenic to A/He mice** "alpha-Terpineol and beta-terpineol injected intraperitoneally to mice were not found to be carcinogenic."

1,8-Cineole (Eucalyptol) (Bhowal and Gopal (2015))

- In a study of the carcinogenic effects of toothpaste constituents including chloroform, eucalyptol, and peppermint oil, eucalyptol was given to groups of 52 male specific pathogen-free CFLP mice at a dose of 8 or 32 mg/kg per day by gavage, 6 days per week for 80 weeks. Control groups of 52 mice were either untreated or received a toothpaste base which lacked chloroform, peppermint, or eucalyptol (vehicle control). Animals were housed four per cage and given food and water *at libitum*. Mice were weighed weekly for the first 6 months and then every 2 weeks during the last 6 months of the study. The consumption of food was noted on a cage-by-cage basis. Animals were observed twice daily and those found dead or in a moribund condition during the study were gross inspected.
- At week 80, animals were killed and organ weights for the kidneys, adrenals, lungs, liver, and spleen were noted. All macroscopically identified tumors were examined histopathologically, along with tissues from the kidneys, liver, lungs and brain.
- No treatment-related changes were reported for the following parameters: food consumed, body weight, organ weights, and clinical signs of toxicity. Necropsy and organ weight measurements showed no treatment-related differences between control and test groups. Histopathological examination revealed **no notable differences between control, test, or vehicle control groups in the incidence or severity of tumors of the kidney, liver, lung, or malignant lymphoma.**
- Studies using the substrain of A mouse strain originating from Walter Heston [A/HE] primary lung tumor model was carried out for carcinogenicity of eucalyptol (12 g/kg/ 8 wk intermittent; Max tolerated dose) but it was observed to be **negative for tumor induction** (Bhowal and Gopal (2015).

Limonene (Jameson (1990) and IARC (1999))

- *Animal carcinogenicity data*
  D-Limonene was tested as a cancer-preventive agent in other experimental models with known carcinogens. It inhibited lung carcinogenesis in mice, preneoplastic stages of colon carcinogenesis in rats and pancreatic carcinogenesis in d-Limonene was tested for carcinogenicity by oral gavage in mice and rats and in several two-stage experiments with multi-organ carcinogens. In the tested animals, limonene significantly increased the incidence of renal tubular tumors (adenomas and carcinomas) and induced atypical renal tubular hyperplasia in male rats only, which normally synthesize α2u-globulin in the liver, but not in female rats or in mice of either sex. It consistently enhanced the incidences of renal tubular tumors and atypical renal tubular hyperplasia initiated by carcinogens in two-stage carcinogenesis assays in male rats of a strain conventionally used in bioassays, but not in a strain that lacks hepatic synthesis of α2u-globulin.

[32] https://echa.europa.eu/de/registration-dossier/-/registered-dossier/22822/7/8 (accessed: 22.03.2021)

D-Limonene was tested as a cancer-preventive agent in other experimental models with known carcinogens. **It inhibited lung carcinogenesis in mice, preneoplastic stages of colon carcinogenesis in rats and pancreatic carcinogenesis in hamsters.**

- *Relevant data*

  d-Limonene is metabolized in humans and experimental animals to a variety of metabolites, including perillic acid and d-limonene-1,2-diol. d-Limonene causes a male rat specific nephrotoxicity resulting from accumulation of the male rat-specific protein α2uglobulin. D-Limonene-1,2-epoxide binds reversibly to α2u-globulin. d-Limonene causes sustained cell proliferation in renal proximal tubular cells, and the dose–response relationships for tumors outcome, enhanced cell proliferation and other histopathological end-points typical of α2u-globulin nephropathy are similar. Female rats, male rats of strains that do not express this protein and other species are not susceptible to the nephrotoxic action of d-limonene.

  The few available data indicate that d-limonene and its 1,2-epoxide metabolite are not genotoxic

- *Evaluation*

  There is inadequate evidence in humans for the carcinogenicity of d-limonene. There is sufficient evidence in experimental animals for the carcinogenicity of d-limonene.

  In making its overall evaluation of the carcinogenicity to humans of d-limonene, the Working Group concluded that d-limonene produces renal tubular tumors in male rats by a on-DNA-reactive mechanism, through an α2u-globulin-associated response. Therefore, the mechanism by which d-limonene increases the incidence of renal tubular tumors in male rats is not relevant to humans. d-Limonene is not classifiable as to its carcinogenicity to humans (Group 3). (Jameson (1990) and IARC (1999)).

Overall, it is demonstrated that TTO and its main component terpinen-4-ol have anticarcinogenic potential. On the other hand, 1,8-cineole and terpineol (TTO terpene compounds), though through i.p. administration, were not carcinogenic in laboratory animals. While limonene induced renal tumors in male rat only, the underlying mechanism is very specific to this species and not relevant to other species and to humans.

The carcinogenicity studies cited cover the following TTO components:

- Terpineol which forms 7.4% of TTO in average (though its study carried out by IP administration). Terpinen-4-ol, which forms 44 % of TTO, is close analogue to Terpineol. Thus, around 51% of TTO components could be covered by the study of Terpineol.
- 1,8-cineole forms 5.2 % of TTO.
- The structural analogy of limonene is comparable to alpha- and gamma-terpinene, alpha-terpinolene, alpha-pinene and sabinene. All these form about 40% of TTO. Thus, around 40% of TTO components could be covered by the studies of Limonene.

In total, the carcinogenicity studies of 1,8-cineole, terpineol and limonene cover > 95% of TTO components. Hence, TTO is unlikely to be a carcinogen.

In conclusion, consumers are exposed to TTO and its components from natural sources and from their use in health care products and as food flavors. However, they are not exposed to TTO/its components from crops treated with TTO, due to the lack of residues on these treated crops. Absorbed TTO is rapidly cleared from the body, there is no potential for bioaccumulation and there is no specific target organ in which TTO (or components) is incorporated after absorption. A genotoxic mode of action for carcinogenicity is highly unlikely since all available *in vitro* and *in vivo* tests for genotoxicity of TTO were negative. Terpineol, 1,8-cineole and limonene do not show carcinogenic potential in publicly available studies which are relevant to humans. In

contrast, it has been demonstrated that TTO and its main component terpinen-4-ol have anticarcinogenic potential. Based on the above, it is very unlikely that TTO has carcinogenic potential.

10.9.2 Comparison with the CLP criteria

From the above, there is no evidence that TTO or its components are carcinogenic in the studies summarized above. Thus a classification for carcinogenicity is not required for TTO.

10.9.3 Conclusion on classification and labelling for carcinogenicity

Not classified – due to inconclusive data.

10.10 Reproductive toxicity

The reproductive toxicity of Tea Tree Oil has been investigated in rats, rabbits and dogs. A two-generation study in rats, is available to investigate the effects of Tea Tree Oil on sexual function and fertility. One developmental toxicity study in rats (oral) and one in rabbits (oral) are also available.

10.10.1 Adverse effects on sexual function and fertility

The effect of Tea Tree Oil on sexual function and fertility has been investigated in a two-generation reproduction study in rats

Table 31: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	NOEL/NOAEL [mg/kg bw/day]	Results	Reliability score	Reference
Two generation study in the rat OECD 416 Oral (gavage) GLP Dose levels: Generation-P: 0, 10, 25 and 50 mg/kg day. Generation-F1: 0, 10, 25 and 38 mg/kg day Treatment related alterations were observed in the reproductive performance at 50 mg/kg bw/day (P generation). Hence, the high dose of 50 mg/kg bw/day was reduced to 38 mg/kg bw/day for the pups selected for F1 generation.	Tea Tree Oil Purity: 10.30 % α-Terpinene, 20.90 % γ-Terpinene, 1.53 % p-cymene and 42.36 % Terpinen-4-ol (in compliance with ISO specification) Vehicle: Groundnut oil Administration: gavage	Reproduction/ offspring NOAEL: 25 mg/kg bw/day	<u>25 mg/kg/day</u> <u>No effects observed</u> <u>38 mg/kg day:</u> ↓pup mean body weight (males + females of F1 generation). ↓Progressive motile sperm (parental F1) <u>50 mg/kg day:</u> ↓No corpora lutea (P) ↓Gestation length (P) ↓Implantations (P) ↓Mean litter size (P) ↓Mean viable litter size (P) ↓Day 4 survival index (P) ↓Male and female fertility indices (P) ↓Sperm motility (P) ↓Cauda epididymal sperm count (P) ↑Percent abnormal sperm (P) *More detailed results are presented in Table 33 – Table 37*	1	Anonymous (2017a)

Table 32: Summary table of repeated dose toxicity studies relevant for toxicity on sexual function and fertility

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Relia-bility score	Reference
28-day feeding study, rats (Wistar) OECD 407 (2008) Non-GLP Dose levels: 0, 62.5, 125, 250 mg/kg bw/day Deviations: not all required organs have been fixed for histopathological examination	Tea Tree Oil Purity: 9.45 % α-Terpinene, 5.67 % 1,8-Cineole, 21.04 % γ-Terpinene, 2.35 % p-cymene and 37.98 % Terpinen-4-ol (in clompliance with ISO specification) Vehicle: Groundnut oil Administration: gavage	NOEL = 62.5 mg/kg body weight/day	<u>At 62.5 mg/kg bw/day</u> <u>No effects observed</u> <u>At 125 mg/kg bw/day</u> • Degenerative changes in testes • Oligospermia • Epididymal cell debris • Pale liver • Hepatocyte vacuolation • ↑Liver weight <u>At 250 mg/kg bw/day</u> ↓ absolute and relative weights of testes and epididymides • Small sized epididymides and testes • Degenerative changes in testes • Aspermia • Pale liver • Hepatocyte vacuolation • Zona fasciculata hypertrophy (adrenals) • ↑Liver weight • ↑Adrenal weight *More detailed results are presented in Table 38 - 40*	2	Anonymous (2010b)

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Relia-bility score	Reference
90-days, feeding, rats (Wistar rats – HsdCpb) OECD 408 GLP Dose levels: 0, 30, 60, 120 mg/kg bw/day	Tea Tree Oil Purity: 9.45 % α-Terpinene, 5.67 % 1,8-Cineole, 21.04 % γ-Terpinene, 2.35 % p-cymene and 37.98 % Terpinen-4-ol (in compliance with ISO specification) Vehicle: Groundnut oil Administration: gavage	Males: NOAEL = 30 mg/kg bw/day Females: NOAEL (= 60 mg/kg bw/day	<u>At 30 mg/kg bw/day</u> <u>No effects observed</u> <u>At 60 mg/kg bw/day</u> ↓ Sperm counts and motility ↑ Percent abnormal sperms <u>At 120 mg/kg bw/day</u> ↓ Sperm counts and motility ↑ Percent abnormal sperms ↓ absolute and relative weights of testes and epididymides -degenerative changes in seminiferous tubules -cell debris in tubular lumen of testes and atrophic appearance -sertoli cell vacuolation -sperm granuloma -cell debris in epidydimal duct lumen • Spleen vacuolation (minimal degree) • Tubular dilatation in kidneys (minimal degree) *More detailed results are presented in Table 41 43*	1	Anonymous (2011b)
90-days, feeding, rats (Wistar rat - Hsd Han) OECD 408 GLP Dose levels: 0, 60 mg/kg bw/day	Tea Tree Oil Purity: 10.3% α-Terpinene, 20.9% γ-Terpinene, 1.36% 1,8-Cineole, 1.53% p-Cymene and 42.36% Terpinen-4-ol. (in compliance with ISO specification) Vehicle: Groundnut oil Administration: gavage	LOAEL = 60 mg/kg bw/d (effects on sperm reversible after recovery period)	<u>At 60 mg/kg bw/day</u> ↓ Sperm counts and motility ↑ Percent abnormal sperms - Sperm granuloma - Oligospermia, - Single cell necrosis, - Luminal cell debris - Degeneration/atrophy of seminiferous tubules More detailed results are presented in Table 44	1	Anonymous (2016a)

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Relia-bility score	Reference
90-days oral, dogs (Beagle) OECD 409 GLP Dose rates: 0, 30, 75/60, 180/120 mg/kg bw/day **(dose reduction from test day** 27 on due to signs of intoxication)	Tea Tree Oil Purity: 9.95% α-Terpinene, 20.35% γ-Terpinene, 4.42% 1,8-Cineole, 1.85% p-Cymene and 41.92% Terpinen-4-ol. (in compliance with ISO specification) Vehicle: Sesame oil Administration: gavage	NOAEL = 30 mg/kg bw/day	<u>At 30 mg/kg bw/day</u> <u>No effects observed</u> <u>At 75/60 mg/kg bw/day</u> ↓ viability and motility of the canine spermatids <u>At 180/120 mg/kg bw/day</u> ↓ viability and motility of the canine spermatids • Clinical signs (starting within 5 minutes after administration and lasting up to 20 minutes) • ↓Body weight/gain (180mg) • ↓Food consumption (180mg) *More detailed results are presented in Table 45 - Table 47*	1	Anonymous (2018a)

Study 1: Anonymous (2017a) Tea Tree Oil: Two generation reproduction toxicity study in Wistar rats, OECD 416, GLP

Tables providing more detailed numerical information are too extensive to be integrated in the summary table above. Therefore they are presented in the following table:

Table: 33 Body weight and food consumption

Parameters		Concentration (mg/kg bwt/day)			
		0	**10**	**25**	**50**
No. of Animals per Concentration		25	25	25	25
Net Body Weight Gain (g)	Males (Day 113)	326.87 ± 39.75	294.31* ± 45.69	309.11 ± 56.71	284.14* ± 45.81
	Females (Day 71)	125.99 ± 14.84	130.93 ± 23.39	135.60 ± 19.35	135.42 ± 26.28
Average Food consumption (g/rat/day)	Males (Week 10)	17.27 ± 1.20	11.92* ± 1.54	13.74* ± 1.80	13.00* ± 1.16
	Females (Week 10)	11.34 ± 0.87	8.32* ± 0.98	9.09* ± 1.00	9.42* ± 0.83
Maternal Body Weight Change during Gestation Period (g)		80.63 ± 14.09	66.53* ± 17.52	68.28* ± 12.83	57.86* ± 17.65
Maternal Food Consumption during Gestation Period (g/rat/day)		14.27 ± 1.29	11.49* ± 1.77	11.66* ± 1.29	12.23* ± 2.19
Maternal Body Weight Change during Lactation Period (g)		-9.50 ± 20.12	-23.96 ± 23.40	-21.20* ± 14.81	-15.06 ± 16.73
Maternal Food Consumption during Lactation Period (g/rat/day)		26,63 ± 4,51	18.88* ± 5.66	20.67* ± 2.98	23.08* ± 6.94
Mean Body Weight of Pups (F1-Generation) during Lactation Period on Day 21 (g)		26,61 ± 5,14	22.24* ± 4.96	22.47* ± 3.91	26.93* ± 5.81

*: Significantly different from the control group (p<0,05)

Table 34: Summary of clinical signs and mortality – Generation: P

Parameters	**Group**	**G1**		**G2**		**G3**		**G4**	
	Dose (mg/kg bwt/day)	**0**		**10**		**25**		**50**	
	Sex	M	F	M	F	M	F	M	F
	No. of rats	25	25	25	25	25	25	25	25
1. GENERAL CONDITION									
Salivation-Slight		0	0	0	0	20	10	23	23
Dehydration-Moderate		0	0	0	0	1	0	0	0
Posture-Recumbent		0	0	0	0	1	0	0	0
2. SKIN AFFECTIONS (Sparse hair loss)		0	1	2	0	1	0	1	0
3. EYE AFFECTIONS		0	0	0	0	0	0	0	0
4. UROGENITAL AFFECTIONS		0	0	0	0	0	0	0	0
5. RESPIRATORY AFFECTIONS		0	0	0	0	0	0	0	0
6. PRE-TERMINAL DEATHS (Total)									
Death during treatment		0	0	0	0	0	0	0	0
Death during gestation		NA	0	NA	0	NA	0	NA	0
Death during lactation		NA	0	NA	1	NA	0	NA	0
Dystocia deaths		NA	0	NA	0	NA	0	NA	0
Moribund sacrifice		0	0	0	0	0	0	0	0
Total mortality		0	0	0	1	0	0	0	0

NA: Not Applicable; M: Male; F: Female

Table 35: Summary of clinical signs and mortality – Generation: F1

Parameters	Group	G1		G2		G3		G4	
	Dose (mg/kg bwt/day)	0		10		25		38	
	Sex	M	F	M	F	M	F	M	F
	No. of rats	25	25	25	25	25	25	25	23
1. GENERAL CONDITION		0	0	0	0	0	0	0	0
2. SKIN AFFECTIONS		0	0	0	0	0	0	0	0
3. EYE AFFECTIONS		0	0	0	0	0	0	0	0
4. UROGENITAL AFFECTIONS		0	0	0	0	0	0	0	0
5. RESPIRATORY AFFECTIONS		0	0	0	0	0	0	0	0
6. PRE-TERMINAL DEATHS (Total)									
Death during treatment		0	0	0	0	0	0	0	0
Death during gestation		NA	0	NA	0	NA	0	NA	0
Death during lactation		NA	0	NA	0	NA	0	NA	0
Dystocia deaths		NA	1	NA	0	NA	0	NA	0
Moribund sacrifice		0	0	0	0	0	0	0	0
Total mortality		0	1	0	0	0	0	0	0

NA: Not Applicable; M: Male; F: Female

Table 36: Summary of the sperm evaluation

	P-Generation			**F1-Generation**		
Concentration (mg/kg bwt/day)	**10**	**25**	**50**	**10**	**25**	**38**
No. of Animals per Concentration	25	25	25	25	25	25
Progressive motile sperms %	—	—	↓(15)	—	—	↓(16)
Motile sperms %	—	—	↓(12)			
Normal sperms %	—	—	↓(14)			
Abnormal sperms %	—	—	↑ (486)			
No. of sperms per cauda epididymis	—	↓(16)	↓(32)	—	—	↓(19)
No of sperms per gram of cauda epididymis	—	—	↓(19)	—	—	↓(18)

↑: Increased ↓: Decreased —: No Change

Values in parenthesis indicate percentage change

Table 37: Summary of survival data of pups, mating and fertility index

Parameters	**Concentration (mg/kg bwt/day)**			
	0	**10**	**25**	**50**
No. of Animals per Concentration	25	25	25	25
Male Fertility Index°	84	76	80	44*
Female Fertility Index°	92	84	84	56*
Mean No. of Corpora Lutea#	12.8	11.8	11.7	9.3*
Mean No. of Implantations#	11.1	10.5	10.1	6.7*
Gestation Length (Days)#	22.77 ± 0.53	22.75 ± 0.55	22.55 ± 0.51	22.45 ± 0.69*
Mean Litter Size#	10.0	8.7	9.0	7.0*
Mean Viable Litter Size	9.9	7.8	8.5	6.7*
Day 4 Survival Index	99.1	94.2*	91.1*	81.1*

*: Significantly different from the control group (p<0,05); #: Compared by Levens, ANOVA and Dunnett's test after transformation ($\sqrt{x + ½}$); °: Compared by 'Z' test

The 2-generation reproduction toxicity study performed according OECD TG 416 and in GLP conditions.

For exposure of P generation Tea Tree Oil was mixed in refined groundnut oil and administered orally by gavage to Wistar rats at the dose levels of 10, 25 and 50 mg/kg bwt/d for the male and female rats of P generation. The vehicle or test item was administered to the male rats once daily at approximately the same time each day for 10 weeks prior to mating. The treatment was continued during mating and after completion of mating process until the necropsy. As in males, females received the vehicle or test item once daily at approximately the same time each day (varied by ± 2 hours) for 10 weeks prior to mating. Treatment was continued through mating, pregnancy and up to the weaning of F1 offspring, after which, parental females were sacrificed. F1 generation offspring were treated from weaning till they were sacrificed after obtaining F2 weanlings.

In P generation the pregnancy occurred in 23 (92%), 21 (84%), 21 (84%) and 14 (56%) out of 25 mated female rats in each group. Three dams in control, five dams at 10 mg/kg bwt/day, four dams at 25 mg/kg bwt/day and fourteen dams at 50 mg/kg bwt/day did not deliver litters. The pre-coital interval was longer (not statistically significant) at 50 mg/kg bwt/day, when compare to control group.

Treatment with TTO at 50 mg/kg bwt/day dose resulted in significantly lower male and female mating and fertility indices, associated with decrease in sperm motility, cauda epididymal sperm counts and increase in percentage of abnormal sperm counts, when compared to vehicle control.

The maternal data such as mean number of corpora lutea and implantations were significantly lower and percentage of pre-implantation loss were significantly higher at 50 mg/kg bwt/day, which in-turn resulted in significantly lower mean litter and viable liter sizes. The mean litter size was 10.0, 8.7, 9.0 and 7.0 in the control, 10, 25 and 50 mg/kg bw/d group. Mean litter size and mean viable litter size was significantly reduced at 50 mg/kg bw/d.

For the F1 generation, the top dose of 50 mg/kg bwt/d was reduced to 38 mg/kg bwt/day for the animals due to adverse effect on fertility in P generation. In F1 generation the pregnancy occurred in 25/25 females(100%), 24/25 females (96%), 24/25 females (96%) and 20/23 (87%) females in the 0 (vehicle control), 10, 25 and 38 mg/kg bwt/day dose groups, respectively. The pre-coital interval and the gestation length (average days to litter) was not altered by treatment at all the doses tested. The maternal data such as mean number of corpora lutea and implantations were comparable to the control group. The mean litter size was comparable in control and treatment groups. Statistically significant decrease in the percentage of progressively motile sperms at 38 mg/kg bwt/day was considered test item-related. This finding was also associated with the lower cauda sperm counts (number of sperms per cauda epididymis and number of sperms per gram of cauda epididymis) at 38 mg/kg bwt/day. However microscopic examination of testes and epididymides did not reveal any associated changes.

Based on the results of this study it is concluded that TTO at dose of 50 mg/kg bw/d significantly affected fertility of rats, apparently of male rats, without inducing alteration of body weight, body weight gain or producing significant adverse effects in other internal organs. The No Observed Adverse Effect Level (NOAEL) for reproductive toxicity is considered to be 25 mg/kg bwt/day, under the test conditions and doses employed.

Anonymous (2010b) Tea Tree Oil: 28-Day Repeated Dose Toxicity Study in Wistar Rats (Non-GLP Study)

Tables providing more detailed numerical information are too extensive to be integrated in the summary table 32. Therefore they are presented in the following:

Table 38: Summary of the significant alterations in organ weights and organ ratios

Sex	Male				Female			
Group No.	**G1**	**G2**	**G3**	**G4**	**G1**	**G2**	**G3**	**G4**
Dose (mg/kg bw/day)	0	62.5	125	250	0	62.5	125	250
No. of rats	6	6	6	6	6	6	6	6
Epididymides								
– Absolute	-	-	-	↓(34)	NA	NA	NA	NA
– Relative	-	-	-	↓(28)	NA	NA	NA	NA
Testes								
– Absolute	-	-	-	↓(43)	NA	NA	NA	NA
– Relative	-	-	-	↓(39)	NA	NA	NA	NA
Liver								
– Absolute	-	-	-	-	-	-	↑(19)	↑(23)
– Relative	-	-	-	↑(14)	-	-	↑(18)	↑(32)
Adrenals - Relative	-	-	-	↑(18)	-	-	-	↑(18)

↑/↓: Statistically significant Increase/Decrease
Values in parenthesis indicate % change when compared to mean value of vehicle control
-: No statistical significance

Table 39: Summary of the gross findings

Sex	Male				Female			
Group No.	**G1**	**G2**	**G3**	**G4**	**G1**	**G2**	**G3**	**G4**
Dose (mg/kg bw/day)	0	62.5	125	250	0	62.5	125	250
No. of rats	6	6	6	6	6	6	6	6
Epididymides – small sized	0	0	0	2	NA	NA	NA	NA
Testes – small sized	0	0	0	2	NA	NA	NA	NA
Liver – Pale, diffuse	0	0	4	1	0	0	0	2

Table 40: Summary of the microscopic changes observed

Sex	Male				Female			
Group No.	**G1**	**G2**	**G3**	**G4**	**G1**	**G2**	**G3**	**G4**
Dose (mg/kg bw/day)	0	62.5	125	250	0	62.5	125	250
No. of rats	6	6	6	6	6	6	6	6
Adrenals								
– hypertrophy-zona fasciculata	0	0	0	0	0	0	0	0
– Minimal	-	-	-	3	-	-	-	2
Testes								
– degenerative changes bilateral	0	0	4	6	NA	NA	NA	NA
– Minimal	-	-	4	3	NA	NA	NA	NA
– Mild	-	-	-	3	NA	NA	NA	NA
Epididymides								
– aspermia	0	0	0	5	NA	NA	NA	NA
– oligospermia	0	0	2	1	NA	NA	NA	NA
– cell debris in lumen	0	0	4	0	NA	NA	NA	NA
Liver								
– hepatocyte vacuolation	0	0	2	6	1	0	1	5
– Minimal	-	-	2	4	1	-	1	5
– Mild	-	-	-	2	-	-	-	-

The study performed according to OECD TG 407 is acceptable with restriction. Tea Tree Oil given for 28 days by gavage at doses 62.5, 125 and 250 mg/kg bw/d did not produce major systemic toxicity besides increased weight of liver, pale liver, vacuolar degeneration of hepatocytes and the degenerative changes in the testes and aspermia/oligospermia in epididymis which were linked with decreased weights of testes and epididymis at 125 and 250 mg/kg bw/d. NOAEL of 62.5mg/kg bw/d can be established based on results of this study.

Anonymous (Anonymous 2011b) Tea Tree Oil: 90-Day Repeated Dose Toxicity Study in Wistar Rats

Tables providing more detailed numerical information are too extensive to be integrated in the summary table 30. Therefore they are presented in the following:

Table 41: Summary the significant alterations ($p \leq 0.05$) in organ weights and organ ratios of male rats

In-life phase	**Treatment**				**Recovery**			
Group No.	G1	G2	G3	G4	G1R		G4R	
Dose (mg/kg bw/day)	0	30	60	120	0		120	
Sacrificed on day	91				15	29	15	29
Testes No. examined	10	10	10	10	10	10	10	10
Absolute	3.787	-	-	-	3.808	3.760	-	2.855↓ (24)
Ratios to body weight	0.970	-	-	-	0.974	9.967	-	0.717 ↓ (26)
Epididymides – No. examined	10	10	10	10	10	10	10	10
Absolute	1.497	-	-	-	1.561	1.521	-	1.154 ↓ (24)
Ratios to body weight	0.382	-	-	-	0.400	0.390	-	0.290 ↓ (26)

↑/↓: Significant Increase/Decrease of Mean values; Values in parenthesis indicate % change
Note: Data subjected to Shapiro-Wilk test, Levene's test, F-test (ANOVA) and Dunnet's 't' test

Table 42: Summary of the gross lesions found in male rats

In-life phase		**Treatment**				**Recovery**			
Group No.		G1	G2	G3	G4	G1R		G4R	
Dose (mg/kg bw/day)		0	30	60	120	0		120	
Sacrificed on day		91				15	29	15	29
Testes	No. examined	10	10	10	10	5	5	10	10
	Small and flaccid	0	0	0	1	0	0	0	4
	Small	0	0	0	3	0	0	0	0
	Flaccid	0	0	0	0	0	0	2	4
Epididymides	No. examined	10	10	10	10	5	5	10	10
	Abcess	0	0	0	6	0	0	4	1

Table 43: Summary of the microscopic changes observed in male rats

In-life phase		**Treatment**				**Recovery**			
Group No.		G1	G2	G3	G4	G1R		G4R	
Dose (mg/kg bw/day)		0	30	60	120	0		120	
Sacrificed on day		91				15	29	15	29
Testes	No. examined	10	10	10	10	5	5	10	10
Degenerative changes - seminiferous tubules		**0**	**0**	**0**	**8**	**0**	**0**	**9**	**8**
	Minimal	0	0	0	4	0	0	3	2
	Mild	0	0	0	2	0	0	4	4
	Moderate	0	0	0	1	0	0	2	2
	Marked	0	0	0	1	0	0	0	0
Sertoli cell vacuolation		**0**	**0**	**0**	**9**	**0**	**0**	**10**	**9**
	Minimal	0	0	0	7	0	0	6	5
	Mild	0	0	0	2	0	0	4	4
Sperm stasis		**0**	**0**	**0**	**0**	**0**	**0**	**4**	**0**
	Minimal	0	0	0	0	0	0	4	0

In-life phase		Treatment				Recovery			
Group No.		G1	G2	G3	G4	G1R		G4R	
Dose (mg/kg bw/day)		0	30	60	120	0		120	
Sacrificed on day		91				15	29	15	29
Epididymides	No. examined	10	10	10	10	5	5	10	10
Sperm granuloma		**0**	**0**	**0**	**4**	**0**	**0**	**6**	**1**
Cell debris in lumen		**0**	**0**	**1**	**7**	**0**	**0**	**9**	**9**
	Minimal	0	0	1	6	0	0	1	2
	Mild	0	0	0	0	0	0	6	5
	Moderate	0	0	0	0	0	0	1	2
	Marked	0	0	0	0	0	0	1	0
Oligospermia		**0**	**0**	**0**	**3**	**0**	**0**	**5**	**6**
Aspermia		**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**
Kidneys	No. examined	10	10	10	10	5	5	10	10
Dilatation of tubules		**0**	**0**	**0**	**3**	**0**	**0**	**0**	**0**
	Minimal	0	0	0	3	0	0	0	0
Spleen	No. examined	10	10	10	10	5	5	10	10
Vacuolation		**0**	**0**	**0**	**5**	**0**	**0**	**0**	**0**
	Minimal	0	0	0	5	0	0	0	0

This 90-day repeated dose toxicity study in rats with Tea Tree Oil was performed according to OECD TG 408 and in GLP conditions. Tea Tree Oil administered by gavage for 90 days (males) or 91 days (females) at doses of 30, 60 or 120 mg/kg bw/day did not induce significant changes in feed consumption, body weight, locomotor activity, hematology, blood coagulation, blood and urine chemistry parameters at any of the doses in either sex. In macroscopic examination no treatment related changes were seen at 30 and 60 mg/kg bw/d, while at 120 mg/kg bw/d only gross lesions were observed in testes (small testes with flaccid appearance, unilateral or bilateral abscess in epididymides). In histopathological examination the degenerative changes were seen at 120 mg/kg bw/d in seminiferous tubules of testes with vacuolisation of Sertoli cell, in epididymides at 120 mg/kg bw/d sperm granuloma, cell debris in lumen, oligospermia (in 3 out of 10 rats) or aspermia (in 1 out of 10 rats) were observed. In other internal organs of males only minimal degree of tubular dilatation in cortical area of kidneys or minimal degree of vacuolation in red pulp area of spleen at 120 mg/kg bw/d were found. In females no treatment related microscopic changes in all organs were observed except for two moribund sacrificed rats at 120 mg/kg bw/d.

In sperm examination no variation was seen in the sperm parameters of rats treated at 30 mg/kg bw/day. At 60 and 120 mg/kg bw/day, significant reduction in the sperm counts and motility were observed and these changes were associated with microscopic changes in the testes (germ cell degeneration and sertoli cell vacuolation) and epididymides (sperm granuloma and oligospermia) only at 120 mg/kg bw/day. Further, significant increase in the percent abnormal (sperms with headless tail, tailless head, bent tail and bent neck) sperms with corresponding decrease in the percent normal sperms was observed.

The results of this study indicate that testes and epididymis are target organs for TTO toxicity, and seminiferous epithelium and sperm cell are more sensitive to toxicity of TTO than somatic cells of rats.

Taking into account the effect in the most sensitive organs the NOAEL for TTO from this study is 30 mg/kg bw/day.

Anonymous (2016a) Tea Tree Oil: 90-Day Repeated Dose Toxicity Study in Wistar Rats

Tables providing more detailed numerical information are too extensive to be integrated in the summary table above. Therefore they are presented in the following:

Table 44: Summary of the clinical pathology investigations, sacrifice and pathology

Gpe No.	Dose [mg/kg bw/day]	No. of rats per group		Clinical pathology investigations			Pathology			Sacrifice on day
		M	F	Haema-tology	Clinical Chemistry	Urina-lysis	Gross pathology	Organ weights	Histo-pathology	
G1	0	10	10	+	+	+	+	+	+	91
G2	60	10	10	+	+	+	+	+	+	91
G1R	0	10[1]	5[1]	+	+	+	+	+	a	147
G1R	0	10[2]	5[2]	+	+	+	+	+	a	175
G1R	0	10[3]	5[3]	+	+	+	+	+	a	203
G2R	60	10[1]	5[1]	+	+	+	+	+	a	147
G2R	60	10[2]	5[2]	+	+	+	+	+	a	175
G2R	60	10[3]	5[3]	+	+	+	+	+	a	203

+: Yes
a: Gross lesions and target organs
[1]: Rats sacrificed after completion of 8 weeks recovery period
[2]: Rats sacrificed after completion of 12 weeks recovery period
[3]: Rats sacrificed after completion of 16 weeks recovery period

The study performed in GLP conditions according to OECD TG 408, however with a major deviation, since only one dose was used, is considered reliable and results can be used for assessment of health hazard caused by TTO. This study is supplementary to the previous one (Anonymous 2011b). Tea Tree Oil administered by gavage for 90 days to female and male rats at dose of 60 mg/kg bw/day did not induce significant changes in food consumption and body weight or clinical parameters in either sex. In histopathological examination the degeneration/atrophy of seminiferous tubules were seen in testes and sperm granuloma/chronic active inflammation, oligospermia, single cell necrosis, luminal cell debris in epididymides. In sperm examination reduced epididymal sperm counts and vas deferens sperm motility with increased abnormal sperms were found in main group exposed at 60 mg/kg bw/day. The pathological changes in testes and epididymides and in sperm examinations observed in rats exposed for 90 days at 60 mg/kg bw/day were no longer seen 8, 12 and 16 weeks after cessation of exposure indicating a complete recovery of damaged organs and tissues.

Anonymous (2018a) Repeated dose 90-Day oral toxicity study of Tea Tree Oil in Beagle dogs - - according to OECD 409, GLP

Tables providing more detailed numerical information are too extensive to be integrated in the summary table above. Therefore they are presented in the following tables :

Table 45: Body weight over recovery period in repeated dose 90-day oral toxicity study

Bodyweight (kg)

Sex: Male		Day(s) Relative to Start Date			
		99	106	113	119
Group 1: Control	Mean	10.45	10.60	10.83	10.70
	SD	0.26	0.29	0.22	0.12
	N	4	4	4	4
Group 4: 180/120 mg/kg	Mean	10.27	10.33	10.53	10.43
	SD	1.62	1.40	1.46	1.59
	N	3	3	3	3
	%Diff	-1.8	-2.5	-2.7	-2.5

Bodyweight (kg)

Sex: Male		Day(s) Relative to Start Date			
		127	134	141	147
Group 1: Control	Mean	10.70	10.50	10.60	10.55
	SD	-	-	-	-
	N	2	2	2	2
Group 4: 180/120 mg/kg	Mean	10.00	9.80	10.20	10.10
	SD	-	-	-	-
	N	1	1	1	1
	%Diff	-6.5	-6.7	-3.8	-4.3

Bodyweight (kg)

Sex: Female		Day(s) Relative to Start Date			
		99	106	113	119
Group 1: Control	Mean	9.65	9.70	9.28	9.85
	SD	1.53	1.54	1.13	1.45
	N	4	4	4	4
Group 4: 180/120 mg/kg	Mean	8.83	8.98	9.18	8.93
	SD	0.97	0.97	1.02	0.91
	N	4	4	4	4
	%Diff	-8.5	-7.5	-1.1	-9.4

Bodyweight (kg)

Sex: Female		Day(s) Relative to Start Date			
		99	106	113	119
Group 1: Control	Mean	9.65	9.70	9.28	9.85
	SD	1.53	1.54	1.13	1.45
	N	4	4	4	4
Group 4: 180/120 mg/kg	Mean	8.83	8.98	9.18	8.93
	SD	0.97	0.97	1.02	0.91
	N	4	4	4	4
	%Diff	-8.5	-7.5	-1.1	-9.4

The summary of food consumption data is not provided due to its extents. Please refer to the orginal study report.

Table 46: Summary of sperm analysis compared to the respective values of the control animals in repeated dose 90-day oral toxicity study

Changes in sperm viability and motility compared to the control group 1 at the end of the treatment period (test week 13)				
Parameter	**Group 1 Control**	**Group 2 30 mg/kg**	**Group 3 75/60 mg/kg**	**Group 4 180/120 mg/kg**
Viability [%]:				
Alive / Dead	83.4 / 16.6	85.5 / 13.5	54.8 / 45.2**	63.1 / 36.9**
Motility [%]:				
Estimated motility	68.8	81.3	20.0**	43.0**
Progressive motility	54.5	61.0	9.7**	29.4**
Non-progressive motility	16.3	23.3	14.3	15.0
Immotility	29.3	15.8	76.0**	55.6**

**: statistically significant at $p \leq 0.01$ (chi^2-test)

Table 47: Body weight measurements (g) in repeated dose 90-day oral toxicity study

Bodyweight (kg)

Sex: Male		Day(s) Relative to Start Date						
		-9 [a]	1 [a1]	8 [a1]	15 [a1]	22 [a1]	29 [a1]	36 [a1]
Group 1: Control	Mean	7.64	7.75	8.11	8.29	8.46	8.75	8.94
	SD	0.67	0.62	0.60	0.62	0.54	0.52	0.51
	N	8	8	8	8	8	8	8
Group 2: 30 mg/kg	Mean	7.63	7.78	8.25	8.43	8.63	9.03	9.25
	SD	1.04	1.01	1.18	1.27	1.27	1.20	1.28
	N	4	4	4	4	4	4	4
	%Diff	-0.2	0.3	1.7	1.7	1.9	3.1	3.5
Group 3: 75/60 mg/kg	Mean	7.50	7.65	7.85	8.28	8.50	8.75	9.10
	SD	0.70	0.70	0.88	0.88	0.88	0.96	0.96
	N	4	4	4	4	4	4	4
	%Diff	-1.8	-1.3	-3.2	-0.2	0.4	0.0	1.8
Group 4: 180/120 mg/kg	Mean	7.61	7.80	7.78	7.29	7.33	7.46	8.21
	SD	0.73	0.63	0.80	0.82	1.04	1.45	0.98
	N	8	8	8	8	8	8	7
	%Diff	-0.3	0.6	-4.2	-12.1	-13.4	-14.7	-8.1

Bodyweight (kg)

Sex: Male		Day(s) Relative to Start Date						
		43 [a]	50 [a]	57 [a]	64 [a]	71 [a]	78 [a]	85 [a1]
Group 1: Control	Mean	9.19	9.34	9.46	9.76	9.96	10.04	10.14
	SD	0.51	0.53	0.50	0.52	0.56	0.54	0.51
	N	8	8	8	8	8	8	8
Group 2: 30 mg/kg	Mean	9.58	9.60	9.63	10.05	10.23	10.40	10.38
	SD	1.47	1.28	1.37	1.42	1.50	1.53	1.53
	N	4	4	4	4	4	4	4
	%Diff	4.2	2.8	1.7	2.9	2.6	3.6	2.3
Group 3: 75/60 mg/kg	Mean	9.13	9.38	9.48	9.73	9.98	10.13	10.10
	SD	0.97	0.90	0.86	0.90	0.91	0.95	0.91
	N	4	4	4	4	4	4	4
	%Diff	-0.7	0.4	0.1	-0.4	0.1	0.9	-0.4
Group 4: 180/120 mg/kg	Mean	8.67	8.66	8.89	9.27	9.60	9.80	9.84
	SD	0.89	0.88	0.89	0.94	1.05	1.13	1.24
	N	7	7	7	7	7	7	7
	%Diff	-5.6	-7.3	-6.1	-5.0	-3.6	-2.4	-2.9

Day(s) Relative to Start Date
91
10.19
0.52
8
10.43
1.53
4
2.3
10.20
0.91
4
0.1
9.93
1.28
7
-2.5

Bodyweight (kg)

Sex: Female		Day(s) Relative to Start Date						
		-10 [a]	1 [a1]	8 [a1]	15 [a]	22 [a]	29 [a]	36 [a]
Group 1: Control	Mean	6.84	6.78	7.18	7.50	7.75	8.10	8.36
	SD	1.06	1.03	1.15	1.21	1.21	1.38	1.33
	N	8	8	8	8	8	8	8
Group 2: 30 mg/kg	Mean	6.58	6.65	7.05	7.30	7.53	7.75	8.00
	SD	1.22	1.28	1.39	1.33	1.24	1.33	1.44
	N	4	4	4	4	4	4	4
	%Diff	-3.8	-1.8	-1.7	-2.7	-2.9	-4.3	-4.3
Group 3: 75/60 mg/kg	Mean	6.83	6.75	7.23	7.48	7.70	7.90	8.00
	SD	1.32	1.53	1.55	1.58	1.54	1.65	1.75
	N	4	4	4	4	4	4	4
	%Diff	-0.2	-0.4	0.7	-0.3	-0.6	-2.5	-4.3
Group 4: 180/120 mg/kg	Mean	6.88	7.11	7.39	7.23	7.35	7.36	7.69
	SD	0.99	0.87	0.95	0.89	1.01	1.03	1.00
	N	8	8	8	8	8	8	8
	%Diff	0.5	5.0	3.0	-3.7	-5.2	-9.1	-8.1

Bodyweight (kg)

Sex: Female		Day(s) Relative to Start Date						
		43	50	57	64	71	78	85
Group 1: Control	Mean	8.44	8.48	8.80	8.79	8.94	9.04	9.23
	SD	1.34	1.47	1.62	1.55	1.53	1.57	1.56
	N	8	8	8	8	8	8	8
Group 2: 30 mg/kg	Mean	8.28	8.45	8.63	8.88	9.00	9.10	9.15
	SD	1.42	1.50	1.48	1.53	1.45	1.45	1.30
	N	4	4	4	4	4	4	4
	%Diff	-1.9	-0.3	-2.0	1.0	0.7	0.7	-0.8
Group 3: 75/60 mg/kg	Mean	8.28	8.35	8.53	8.73	8.80	8.93	8.88
	SD	1.84	1.81	1.65	1.71	1.62	1.60	1.72
	N	4	4	4	4	4	4	4
	%Diff	-1.9	-1.5	-3.1	-0.7	-1.5	-1.2	-3.8
Group 4: 180/120 mg/kg	Mean	7.89	8.11	8.34	8.51	8.68	8.85	8.73
	SD	1.12	1.06	1.17	1.30	1.30	1.31	1.14
	N	8	8	8	8	8	8	8
	%Diff	-6.5	-4.3	-5.3	-3.1	-2.9	-2.1	-5.4

Day(s) Relative to Start Date
91
9.05
1.50
8
8.80
1.35
4
-2.8
8.73
1.42
4
-3.6
8.51
1.23
8
-5.9

[a] - Anova & Dunnett [a1] - Anova & Dunnett(Log)

The 90-day study of repeated dose toxicity in dogs performed according to OECD TG 409 and in GLP conditions is acceptable and results can be used for assessment of health hazard and risk caused by TTO. Tea Tree Oil administered by gavage to Beagle dogs (4 animals/sex/dose level) for 90 days at doses of 30, 60 or 120 mg/kg bw/day did not affect the feed consumption, body weight, hematological, biochemical and urine parameters at any of the doses in either sex. The highest dose was 180 mg/kg for first 4 weeks, but it was reduced to 120 mg/kg bw/d due to excessive toxicity. The mid dose was also reduced after first 4 weeks from 75 mg/kg to 60 mg/kg bw/d. In macroscopic and histopathological examinations no treatment related changes were seen at 30, 60 and 120 mg/kg bw/d after termination of exposure and at the end of 4-week and 8-week treatment free recovery periods, however size of right and left testicles were reduced in dogs given the highest dose in comparison with those of control animals. Exposure of male dogs to TTO at doses 75/60 and 180/120 mg/kg bw/day did not affect morphology and mean number of spermatozoa in ejaculate, but led to a decrease of percentage of alive spermatozoa and percentage of motile spermatozoa. No changes in viability or motility, in comparisons to the controls, were noted in dogs exposed at 30 mg/kg bw/d.

At the end of 4 and 8 weeks recovery periods the percentage motile and viable of spermatozoa in the group 180/120 mg/kg bw/day was not different from the concurrent control indicating a full recovery from alterations induced by TTO. The results of this study indicate that male reproductive organs are target organs for TTO toxicity, and sperm cell are more sensitive to toxicity of TTO than somatic cells of dogs. NOAEL in dogs for TTO established based on results of this study is 30 mg/kg bw/day.

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

In the 2-generation study treatment with TTO at 50 mg/kg bwt/day dose resulted in P generation significantly lower male and female mating and fertility indices, associated with decrease in sperm motility, reduced cauda epididymal sperm counts and increase in percentage of abnormal sperm counts, when compared to vehicle control. The mean number of corpora lutea and implantations were significantly lower and percentage of pre-implantation loss were significantly higher at 50 mg/kg bw/day, which in-turn resulted in significantly lower mean litter and viable liter sizes. These effects were not observed at dose of 10 and 25 mg/kg bw/d.

The highest dose 50 mg/kg bw/d was reduced to 38 mg/kg bw/d for animals selected for F1 generation due to adverse effect on fertility. In F1 generation statistically significant decrease in the percentage of progressively motile sperms at 38 mg/kg bwt/day was considered test item-related. This finding was also associated with the lower cauda sperm counts (number of sperms per cauda epididymis and number of sperms per gram of cauda epididymis) at 38 mg/kg bwt/day.

Parental toxicity was limited to slight salivation for short time after dosing of TTO at 25 and 50 mg/kg. No treatment related changes of body weight and organ weight were observed. No treatment related histopathological changes were observed in parental animals (males and females) as well as in pups of P and F1 generations at all dose levels tested. The results of the 2-generation study indicate that TTO at dose of 50mg/kg bw/d by gavage have adverse effect on sperm count and sperm motility leading to reduced fertility of rats, while at dose 38 mg/kg bw/d TTO affects the motility and sperm counts without reducing fertility index in the exposed group. The No Observed Adverse Effect Level (NOAEL) for reproductive toxicity is considered to be 25 mg/kg bwt/day under the test conditions.

The adverse effect of TTO on testes and/or sperm count and motility was also noted in the repeated dose toxicity studies in rats given a test substance by gavage for 28 days at dose of 250 mg/kg bw/d, in rats given a test substance by gavage for 90 days at dose of 60 and 120 mg/kg, in dogs given a test substance by gavage for 90 days at dose of 60/75 mg kg/bw/d and 120 mg/kg bw/d. These effect were not observed in rats and dogs given for 90 days by gavage TTO at dose of 30 mg/kg, so this dose level cab be taken as No Observed Adverse Effect Level (NOAEL)

10.10.3 Comparison with the CLP criteria

The adverse effects of TTO on fertility, testes, epididymides and sperm observed in two species (rats and dogs) in four acceptable studies at dose levels inducing slight or moderate general systemic toxicity provide some evidence meeting the classification criteria for reproductive toxicity of TTO in animals. It is noted that such effects were not reported in humans exposed to components of TTO at relatively high doses with food, although no targeted epidemiological studies were done, therefore there is some doubt whether these effects observed in animals are relevant for humans. Taking the above uncertainty into account DS is of the opinion that TTO warrants classification to subcategory Repr. 2 with hazard statement H361f - Suspected of damaging fertility. The highest dose level at which these effects were not observed in 2-generation study was 25 mg/kg bw/d, which considered as No Observed Adverse Effect Level (NOAEL) for reproductive toxicity.

10.10.4 Adverse effects on development

The potential of Tea Tree Oil to adversely affect development has been investigated in rats and rabbits by the oral route.

Table 48: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reliability score**	**Reference**
Prenatal Developmental Toxicity Study in the rat OECD 414 GLP Wistar rats – HsdHan Females 24/group (owing to severe clinical signs and mortality doses were reduced)	Tea Tree Oil Purity: 8.18 % α-Terpinene, 1.80 % 1,8-Cineole, 14.23 % γ-Terpinene, 3.86 % p-cymene and 41.73 % Terpinen-4-ol (in compliance with ISO specification) Vehicle: refined peanut oil Administration: gavage Dose rates: 0, 75, 150 and 300 mg/kg/day and 0, 30, 60 and 120 mg/kg/day.	<u>60 mg/kg day:</u> ↓Maternal body weight ↓Maternal food intake <u>120 mg/kg day:</u> ↓Maternal body weight ↓Maternal food intake ↓Fetal weight <u>150 mg/kg day:</u> Clinical signs Incidence of mortality ↓Maternal body weight ↓Maternal food intake <u>300 mg/kg day:</u> Clinical signs Incidence of mortality ↓Maternal body weight ↓Maternal food intake ↑Resorptions *More detailed results are presented in* Table *49 - Table 50* NOAEL, maternal toxicity: 30 mg/kg/day NOAEL, fetal toxicity: 60 mg/kg/day	1	Anonymous (2012a)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reliability score	Reference
Prenatal Developmental Toxicity Study in the rabbit Oral (gavage) OECD 414 GLP New Zealand white rabbits 24/group	Tea Tree Oil Purity: 9.95% α-Terpinene, 20.35% γ-Terpinene, 4.42% 1,8-Cineole, 1.85% p-Cymene and 41.92% Terpinen-4-ol. (in compliance with ISO specification) Vehicle: refined peanut oil Administration: gavage Dose rates: 0, 15, 30, and 75 mg/kg/day	<u>75 mg/kg day:</u> ↑Post implantation loss *More detailed results are presented in Table 51 - Table 52* NOAEL, maternal toxicity: 75 mg/kg/day NOAEL, fetal toxicity: 30 mg/kg/day NOAEL teratogenicity: 75 mg/kg/day	1	Anonymous (2018b)
Prenatal Developmental Toxicity Study in the rat Oral (gavage) OECD 414 GLP Wistar rats – HsdHan:WIST Up to 27 females/dose level	Melaleuca alternifolia, ext., Purity: 100% Content of terpinen-4-ol: 37% 0 mg/kg bw/day Group 1. Control (vehicle only - PEG 400). 20 mg/kg bw/day Group 2. Low dose. 100 mg/kg bw/day Group 3. Mid dose. 250 mg/kg bw/day Group 4. High dose. Vehicle: Polyethylene glycol 400 (PEG 400) Exposure: From days 5 to 19 of gestation (GD 5 to GD 19) (Daily treatment by oral gavage 7 days/week, at a similar time each day.)	Maternal animals: NOAEL: 20 mg/kg bw/day based on: (test mat.) Adverse effects at 100 and 250 mg/kg bw/day comprised clinical signs, reduced food consumption and reduced weight loss gains (with mortality at the high dose). Fetuses: NOAEL: 20 mg/kg bw/day based on: (test mat.) Reductions in foetal body weight were seen at 100 and 250 mg/kg bw/day. Increases in external and skeletal malformations were also seen in foetuses from the high dose group. All effects were secondary to maternal toxicity. Overall developmental toxicity: yes Lowest effective dose / concentration: 100mg/kg bw/day. Relation to maternal toxicity: Reproductive effects as a secondary non-specific consequence of other toxic effects.	1	ECHA dissemination site (study report 2011)[33]

[33] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/9/3

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

The developmental toxicity of Tea Tree Oil has been investigated in rats (oral, gavage) and rabbits (oral, gavage).

Study 1, Anonymous, (2012a), Prenatal developmental toxicity study of Tea Tree Oil in Wistar Rats by oral route, OECD 414, GLP.

Reliability statement: The study is conducted in accordance with OECD 414. It meets the validity requirements as set out in the test guideline, shows only minor deviations and the test substance reflects the ISO 4730:2004. Hence, the study is considered reliable without restictions (reliability score: 1).

In the prenatal developmental toxicity study (Anonymous 2012a) in rats TTO was administered orally by gavage on GD 5- GD19 to pregnant Wistar rats at the initial tested doses of 75, 150 and 300 mg/kg/day and at the reduced doses of 30, 60 and 120 mg/kg/day. The doses were reduced on GD 8 (third day of treatment) due to mortality, at a dose of 300 mg/kg.

Maternal toxicity: At initial doses 150 and 300 mg/kg bw/d TTO induced clinical signs of dullness and incidence of mortality at 300 mg/kg bw/d, but no clinical signs were observed at doses 30, 60 and 120 mg/kg bw/d. Body weight gain and food consumption were reduced at 150/60 and 300/120 mg/kg bw/d. No gross visceral pathology was observed at necropsy in any treated groups.

Developmental toxicity: Mean number of corpora lutea, implantation, early resorption, late resorption, pre-implantation loss, post-implantation loss were not affected by TTO at any dose. Number (11 and 12) and percentage (47.8 and 57.1%) of dams with any resorption were higher at dose 150/60 and 300/120 mg/kg bw/d than in control group (6 and 25%).

Total number of life fetuses, mean litter size were unaffected, but the mean weight of fetuses in the groups 150/60 and 300/120 mg/kg bw/d were significantly reduced by respectively 4.6% and 15%. No major external, visceral or skeletal malformations were observed an no effect of TTO on incidence of minor external, visceral or skeletal anomalies was found in any exposed group. Increased incidence of delayed ossification of various bones was observed in 150/60 and 300/120 mg/kg bw/d. The No Observed Adverse Effect Level (NOAEL) for maternal and developmental toxicity of 30/75 mg/kg bwt/day can be derived due to mortality at dose of 300 mg/kg and reduced body weight gain and food consumption of dams and delayed ossification in foetuses at 150/60 and 300/120 mg/kg bw/d. The effects observed are not meeting classification criteria for developmental toxicity

Table 49: Body weights, body weight gain and food intake during a prenatal developmental toxicity study in the rat treated with Tea Tree Oil

Concentration (mg/kg Bwt/day)		**0**	**75/30**	**150/60**	**300/120**
No. of dams		24	23	23	21
Maternal body weight (g)					
Days of gestation	0	224.83 ± 15.96	223.72 ± 14.92	226.05 ± 15.40	225.66 ± 15.04
	3	233.87 ± 17.92	233.56 ± 18.16	235.71 ± 17.94	236.06 ± 17.90
	5	238.66 ± 18.14	239.00 ± 20.52	241.34 ± 20.95	241.75 ± 19.46
	8	244.78 ± 19.22	243.71 ± 22.73	237.08 ± 21.14	236.74 ± 21.52
	11	256.94 ± 21.03	257.99 ± 24.42	252.55 ± 21.22	248.67 ± 20.42
	14	269.40 ± 20.59	270.13 ± 25.15	262.00 ± 20.10	255.62 ± 22.06
	17	291.96 ± 23.50	293.50 ± 26.49	283.32 ± 22.48	274.30 ± 21.53↓
	20	317.11 ± 25.61	323.14 ± 28.39	308.11 ± 25.76	291.32 ± 25.37↓
Corrected Bwt. gain		18.24 ± 9.21	20.05 ± 13.04	9.33 ± 9.07↓	-4.40 ± 12.15↓
Body weight gain					
Period	Pre-treatment (days 0 – 5)	13.84 ± 5.70	15.28 ± 7.87	15.29 ± 7.46	16.10 ± 6.12
	Treatment (days 5 – 20)	78.45 ± 10.99	84.13 ± 13.04	66.77 ± 14.58↓	49.57 ± 15.40↓
	Throughout gestation (days 0 – 20)	92.28 ± 13.20	99.42 ± 17.12	82.06 ± 15.24	65.67 ± 16.70↓
Food intake (g/day/rat)					
Period (Days of gestation)	0 - 3	15.31 ± 2.82	16.86 ± 3.22	16.52 ± 1.62	16.70 ± 2.08
	3 - 5	16.68 ± 2.54	19.98 ± 3.61	19.09 ± 2.34	19.40 ± 2.42
	5 - 8	13.45 ± 2.52	13.12 ± 2.56	10.35 ± 3.27↓	11.63 ± 3.47
	8 - 11	14.44 ± 2.13	15.12 ± 2.64	13.72 ± 2.30	12.42 ± 2.43↓
	11 - 14	16.59 ± 1.74	16.86 ± 2.22	15.28 ± 1.87	13.39 ± 2.91↓
	14 - 17	16.25 ± 2.15	16.42 ± 2.26	14.74 ± 1.92	12.90 ± 1.96↓
	17 - 20	15.66 ± 2.44	16.19 ± 2.18	14.67 ± 2.15	12.05 ± 2.90↓

↑/↓: Significant Increase/Decrease of Mean values ($p \leq 0.05$)

Table 50: Litter data during a prenatal developmental toxicity study in the rat treated with Tea Tree Oil

Concentration (mg/kg Bwt/day)	**0**	**75/30**	**150/60**	**300/120**
No. of litters examined	24	23	23	21
No. of fetuses examined	134	134	126	118
FETUS ANOMALIES (Incidence and %)				
External observations				
Normal variant	- NIL -			
Minor anomalies				
Haemorrhagic patch on dorsal thoracic spine	0	1 (0.75%)	0	0
Fore limb flexed at wrist (+) (Rt/Lt/B)	0	0	0	1 (0.85%)
Small fetus	0	0	0	1 (0.85%)
Major malformations	- NIL -			
Visceral observations				
Normal variant				
Umbilical artery displaced	7 (5.22%)	6 (4.51%)	7 (5.51%)	7 (5.93%)
Liver median lobe extra lobation	1 (0.76%)	3 (2.26%)	2 (1.57%)	1 (0.85%)
Kidney renal pelvis dila. (Rt/Lt/B) (+)	1 (0.75%)	1 (0.75%)	0	0
Minor anomalies	- NIL -			
Major malformations	- NIL -			
NORMAL VARIANT PARAMETERS FOR WITH SIGNIFICANT ALTERATIONS ($p \leq 0.05$) **IN THE INCIDENCES** (%)				
Delayed skeletal ossification				

	Stern: # 5	2.24	2.26	11.02↑	16.95↑
	Stern: # 5, 6	0.75	0.00	6.30↑	16.95↑
	Cervical centra 2/7	23.88	15.04	15.75	6.78 ↓
	Cervical centra 6/7	13.43	10.53	26.77↑	36.44↑
	Cervical centra 7/7	0.75	0.00	4.72	9.32↑
	Caudal vertebral centra 1/4	17.16	12.78	24.41	60.17↑
	Caudal vertebral centra 2/4	0.00	0.00	5.51↑	20.34↑
	Caudal vertebral arch 1/2	11.94	6.77	16.54	57.63↑
	Forelimb metacarpal 1/4	43.28	44.36	69.29↑	89.83↑
	Forelimb proximal phalange 1/2	2.99	12.78↑	3.94	0.85
	Forelimb proximal phalange 2/2	49.25	54.89	84.25↑	98.31↑
	Forelimb distal phalange 1/4	14.18	11.28	35.43↑	39.83↑
	Hind limb distal phalange 5/5	11.19	3.01↓	15.75	30.51↑
Incomplete/poor ossification					
	Frontal, parietal and interparietal	0.00	0.00	0.00	4.24↑
	Stern: #3	0.00	0.00	0.00	4.24↑
	Stern: #4	0.00	0.00	1.57	8.47↑
	Stern: #6	32.84	24.06	38.58	61.86↑

↑/↓: Significant Increase/Decrease of Mean values ($p \leq 0.05$)

Study 2, Anonymous, (2018b), Tea Tree Oil: Embryo-fetal developmental toxicity study by oral gavage in New Zealand White rabbits, OECD 414, GLP.

Reliability statement: The study is conducted in accordance with OECD 414. It meets the validity requirements as set out in the test guideline, shows only minor deviations and the test substance reflects the ISO 4730:2004. Hence, the study is considered reliable without restictions (reliability score: 1).

In the second study, pregnant rabbits were treated orally by gavage at dose levels of 15, 30 and 75 mg/kg/day from GD 6 to 28.

Rabbits were observed for clinical signs, morbidity, mortality, body weight changes and food consumption. Caesarean section was performed for all the surviving rabbits on GD 29 and dams were examined for gross pathological changes. The uterus was removed by laparotomy, weighed and the contents were examined for number of implantation sites, early and late resorptions and number of fetuses. The number of corpora lutea in ovaries was counted. All the fetuses were sexed, weighed and examined for external malformations. All the live fetuses were examined for visceral and skeletal variations and malformations.

There was no mortality, clinical signs or gross necropsy findings in dams at any of the doses tested.

The group mean maternal body weights during the different days of gestation were comparable to the vehicle control group at all tested dose levels. However, as compared to the control, statistically significant decrease in net body weight gain during GD 6 – 9 was observed at 30 and 75 mg/kg/day. At 30 mg/kg/day, there was a 40 to 42% decrease in mean body weight gain during GD 6 to 29 and 0 to 29 respectively. The decrease in body weight gain was statistically not significant. At 75 mg/kg/day there was significant decrease in body weight gain during treatment period GD 6 - 29 and for entire gestation period 0-29 which was 64 % and 54%, respectively. The decrease in body weight was considered non-adverse as the corrected body weight gain was comparable to vehicle control.

As compared to vehicle control, significant reduction in mean food consumption was observed following treatment at 30 and 75 mg/kg/day dose groups during intermittent periods of GD 6 to 9, 9 to 12, 12 to 15, 15 to 18, 18 to 21, 21 to 24, 24 to 27 and 27 to 29 which was approximately 23 % to 45 % at 30 mg/kg/day and 28% to 63% at 75 mg/kg/day. Also during treatment period GD 6 - 29 and for entire period of gestation GD 0 - 29 there was significant reduction in food consumption which was 28 to 36% at 30 mg/kg/day and 34 to 43% for 75 mg/kg/day.

Table 51: Body weight gain, Food consuption and maternal data during the total gestation period (days 0-29) in a developmental toxicity study in rabbits with Tea Tree Oil

Dose (mg/kg/day)	**0**	**15**	**30**	**75**
No. of Pregnant rabbits	21	20	21	21
Mean body weight gain (kg)				
Pre-treatment period (d 0-6)	0.102 ± 0.09	0.051 ±0.07	0.065 ± 0.11	0.076 ± 0.07
Treatment period (d 6-29)	0.310 ± 0.19	0.284 ± 0.17	0.181 ± 0.20	0.113* ± 0.26
Total gestation period (d 0-29)	0.412 ± 0.21	0.335 ± 0.27	0.246 ± 0.25	0.189* ± 0.29
Corrected Body wt gain (kg)	-0.0036 ± 0.21	-0.043 ± 0.20	-0.137 ± 0.16	-0.199 ± 0.22
Food consumption				
Pre-treatment period (d 0-6)	147.56 ±18.06	154.32 ± 15.75	150.90 ± 14.23	145.86 ± 18.43
Treatment period (d 6-29)	130.14 ± 14.03	123.45 ± 19.26	82.71* ± 24.92	73.98* ± 24.21
Total gestation period (d 0-29)	133.75 ± 12.28	129.84 ± 15.81	96.82* ± 20.56	88.85* ± 20.96
Maternal Data (mean data)				
Gravid Uterine Weight (g)	346.04 ± 114.62	327.41 ± 94.88	317.54 ± 95.02	311.62 ± 110.41
No. of Corpora lutea	8.38 ± 1.66	7.80 ± 1.58	8.19 ± 1.29	8.90 ± 1.73
No. of Impantations	6.52 ± 2.18	6.25 ± 1.89	6.24 ± 2.00	6.76 ± 2.23
No. of Early Resorptions	6.52 ± 2.18	6.25 ±1.89	6.24 ± 2.00	6.76 ± 2.23
No. of Late Resorptions	0.24 ± 0.54	0.30 ± 0.47	0.33 ± 0.48	0.90 ± 1.79
No. of Pre-implantation Loss	1.86 ± 1.31	1.55 ± 1.32	1.95 ± 1.28	2.14 ± 1.31
No. of Post-implantation Loss	0.52 ± 0.81	0.65 ± 0.67	0.76 ± 0.89	1.76* ± 1.84
Dams with any Resorption	8	11	11	15
Dams with all Resorption	0	0	0	1
Maternal data (% per litter)				
Early resorptions	3.45 ± 7.92	6.31 ± 11.04	6.43 ± 10.41	14.30 ± 24.38
Late resorptions	4.61 ± 7.91	5.85 ± 11.99	6.78 ± 11.26	10.72 ± 14.58
Pre-implantation loss	23.87 ± 19.62	20.15 ± 17.19	25.00 ± 17.94	25.17 ± 16.77
Post-implantation Loss	8.06 ± 13.63	12.16 ± 14.74	13.22 ± 18.37	25.02 ± 23.87
Implantation index	76.13 ± 19.62	79.85 ± 17.19	75.00 ± 17.94	74.83 ± 16.77

*: Significantly different from the control group; Corrected Body wt gain = carcass weight - body weight on day 6

The maternal parameters comprising of gravid uterine weight, mean number of corpora lutea, implantations, early and late resorptions, pre and post implantation loss and dams with resorptions at the tested doses of 15 and 30 mg/kg/day treated groups were statistically comparable to the vehicle control group. At 75 mg/kg/day there was a significant increase in post implantation loss and this increase was considered treatment related as the value was higher than historical data.

Gross evaluation of placenta did not reveal any findings in any dams at any tested dose levels.

Table 52: Summary of Litter data

Parameters	**Group No.**	**G1**	**G2**	**G3**	**G4**
	Dose (mg/kg/day)	**0**	**15**	**30**	**75**
	No. of Pregnant rabbits	**21**	**20**	**21**	**21**
No. of litters		21	20	21	20
Total No. of fetuses		126	112	115	105
Mean litter size		6.0	5.6	5.5	5.0
Dead fetuses	Total No.	0	0	0	0
	%	0	0	0	0
Live fetuses	Total No.	126	112	115	105
	Mean weight (g) ± SD	38.84 ± 5.00	38.71 ± 4.98	37.71 ± 4.88	35.13 ± 6.33
Live male fetuses	Total No.	68	59	56	55
	Mean weight (g) ± SD	38.62 ± 4.46	39.55 ± 5.05	38.39 ± 5.35	35.97 ± 5.93
Live female fetuses	Total No.	58	53	59	50
	Mean weight (g) ± SD	38.32 ± 5.45	37.67 ± 5.93	36.37 ± 4.96	33.03 ± 7.31
Sex Ratio - Male: Female		1:0.85	1:0.9	1:1.05	1:0.91
(Percentage of number of males)		(54%)	(53%)	(49%)	(52%)

The litter parameters comprising total number of fetuses, mean fetal weight and number of live fetuses at all the treated groups were statistically comparable to the vehicle control group.

External, visceral and skeletal examination of fetuses revealed no signs of teratogenicity or developmental toxicity.

There were no gross pathological changes in any animal at any dose levels.

The post implantation loss observed within the second study (Anonymous, 2018b) at the highest dose tested can be considered as a consequence of an increased number of late resorptions. Even if a dam with a total loss due to early resorptions was considered by the study author as an outlier, the late resorptions still remain significantly increased while significance of post-implantation loss itself decreases.

In this study it was further reported that in the mid (30 mg/kg bw/day) and high (75 mg/kg bw/day) dose groups the food consumption of the dams was significantly decreased.

Compared to the animals of the control group, the TTO treated animals ate, in a dose dependent manner, significantly less from the sixth day of pregnancy on. Especially between days 6 - 18 (the critical time frame of organogenesis) only about half of the control values was consumed. Since TTO was administered by gavage, the effect cannot simply be attributed to reduced palatability. It can be assumed that consequently an energy deficiency situation has occurred in the dam, which in turn can have negative effects on the development of the fetus.

Concomitantly, as compared to the control, statistically significant decrease in net body weight gain during GD 6 – 9 was observed at 30 and 75 mg/kg/day. At the end of the treatment, all dosed animals showed weight loss (negative net body weight gains which is the terminal BW corrected for uterine weight). Clearly visible in the mid and high dose animal groups, though not significant anymore.

In this study peanut oil was used as the vehicle. Peanut oil can be considered as a vehicle with high caloric content that may cause an animal to consume less food with minimal or no net effect on body weight gain. This is likely the reason that the significant reduction of food consumption does not lead to a clear significant weight loss of the dams.

It has already been described several times that a decrease in maternal net body weight and concomitant reduction in food consumption usually indicate systemic toxicity.

Studies of dietary restrictions have demonstrated that reductions in food consumption of as little as 10% of the normal total dietary intake may be associated with increased prenatal death, dysmorphogenesis and/or growth retardation. Therefore, reduced maternal food intake may be an indication not only for maternal toxicity but also of secondary insult to the developing progeny.

Study 3, ECHA dissemination site, study report 2011, Tea Tree Oil: Oral Gavage Developmental Toxicity Study in the Hannover Wistar Rat, OECD 414, GLP.

A GLP compliant developmental toxicity study was conducted with Tea Tree Oil (TTO) in naturally mated, assumed pregnant Hannover Wistar female rats according to OECD Test Guideline 414, to evaluate the effect on dams and developing conceptuses after oral (gavage) administration during pregnancy. A control group which received PEG 400 only and three groups treated with TTO formulated in PEG 400 at 250, 100 and 20 mg/kg bw/day were included in the study. TTO formulated in PEG 400 was administered daily from gestation day (GD) 5 to GD19, where GD0 was considered the day of mating. Caesarean section and maternal necropsy with macroscopic examination were performed on GD20 in all the females surviving to termination. Placentas

and fetuses were examined macroscopically and fetal body weight was measured. The gender of each fetus was determined. Thereafter, approximately half of each litter was subjected to a visceral examination and the remaining fetuses were processed for skeletal examination.

At 250 mg TTO/kg bw/day, mortality occurred in 7/27 females between GD8 and GD11. Clinical signs in animals that died included noisy respiration, decreased activity and/or piloerection. Clinical signs in surviving animals included decreased activity, hunched back position, noisy respiration, piloerection, red spots on the tail and/or soft feces. Treatment at 100 mg/kg bw/day resulted in no mortality but clinical signs such as noisy respiration, decreased activity, hunched back position, red spots and/or soft feces were noted in 13/26 females. At 20 mg TTO/kg bw/day, there was no mortality. Noisy respiration or soft feces were occasionally noted, but were considered not to be toxicologically relevant.

Severely reduced maternal body weight gain (-20% and -45% respectively, when compared to control) and food consumption were noted at 100 and 250 mg TTO/kg bw/day.

In females treated at 250 mg TTO/kg bw/day, bilateral enlarged adrenals were observed in all animals found dead and in 6/20 of animals that survived until scheduled necropsy. This finding was attributed to treatment. A single animal in the mid dose group also had bilateral enlarged adrenals.

At 250 mg TTO/kg bw/day, there was a higher number of late embryonic deaths and consequently postimplantation loss, leading to an overall higher total intrauterine mortality. Post-implantation losses were unchanged in the low and mid treatment groups. Post-implantation mortality was considered secondary to maternal toxicity.

Statistically lower mean gravid uterine weight was noted at 250 mg TTO/kg bw/day and lower terminal mean body weights when corrected for the gravid uterine weight, were noted at 100 and 250 mg TTO/kg bw/day. The corrected mean body weight gains were lower than the controls in the two highest dose groups. These adverse effects were considered to be related to TTO administration.

Most fetuses were viable and no effects related to TTO were noted in the mean number of viable fetuses/ group, or their sex distribution. The sex ratios were similar in the control and treated groups when evaluated per litter.

Adverse effects were noted in mean fetal weights at 100 and 250 mg TTO/kg bw/day, with a doserelated pattern. The effects on fetal body weight were related to intrauterine growth retardation. External abnormalities such as local edema in the cervical area, generalized edema or short maxilla were noted in the high dose group.There was no statistically significant difference from control in the number of visceral malformations. A statistically higher number of visceral variations were noted in the 250 mg/kg bw/day dose group. These included dilated brain ventricles and displaced gonads associated with the intrauterine growth retardation were noted. In addition, variations such as small nasal conchae, close origin of brachiocephalic and carotid, dilated ureter or dilated renal pelvis were statistically increased at 250 mg/kg bw/day.

A statistically higher incidence of skeletal malformations unrelated to intrauterine growth retardation was noted in the 250 mg/kg bw/day group. This included displaced rib cartilages at the sternum, malformed vertebrae, and/or short, bent scapula, humerus or femur. Statistically higher numbers of skeletal variations, secondary to maternal toxicity, were noted at 100 and 250 mg/kg bw/day.

In summary, severe maternal toxicity was seen in dams from the 100 and 250 mg/kg bw/day groups, as evidenced primarily by clinical signs, reduced food consumption and reduced weight loss gains (and mortality at the high dose). Fetal abnormalities seen in the 100 and 250 mg/kg bw/day groups were secondary to maternal toxicity. The NOAEL for Tea Tree Oil for developmental toxicity (secondary to severe maternal toxicity) was 20 mg/kg bw/day. The NOAEL for maternal toxicity was 20 mg/kg bw/day.

Within the developmental toxicity study in rats (Anonymous , 2012a) there were no relevant findings other than maternal toxicity (reduced body weights and food intake). An increased number of resorptions occurred, however only at extremely high doses (300 mg/kg bw/day) in parallel with severe maternal toxicity and increased mortality.

Furthermore, there are indications that TTO, if administered by gavage, shows pharmakokinetic (and then pharmacodynamic) properties other than after dietary administration: Short-term toxicity tests conducted with Tea Tree Oil revealed detrimental effects on sperm count and motility, at higher doses also linked to microscopical changes in tissue. These effects were demonstrated to be reversible within max. 8 weeks following exposure. It should be noted however, that all these studies, like the present study, were conducted with gavage as method of application, since most components of Tea Tree Oil have extremely high vapour pressure which makes dosing via mixing into food very difficult. However, data is available for bicyclic monoterpenes (α-Terpineol, a constituent of Tea Tree Oil and very similar to its main component Terpinen-4-ol) where reproductive studies were conducted both via gavage and via diet administration. It was demonstrated that after dietary administration of α-Terpineol sperm damage did not occur. Pharmacokinetic analysis confirmed that oral gavage at high doses clearly resulted in much higher systemic exposure than expected, leading to biologically non-relevant effects that should not be considered for classification purposes (for details please refer to 10.10.2). Assuming that the unexpected high systemic exposure is the trigger of sperm damage, it is not unlikely that comparably this overexposure would have a negative impact on the already fragile developmental course, additionally to the affection by maternal food and energy deficiency.

Then the converse conclusion would also be admissible, that after a dietary administration, which is the more relevant one for humans, the degree of late resorption would be lower, if not completely absent.

Overall, the increase in post-implantation loss at the highest tested dose is due to an increased number of late resorptions. This kind of developmental impairment might be caused by reduced food consumption, especially if in parallel the net weight gains of the dams were reduced indicating maternal toxicity. Peak concentrations of TTO in blood after gavage administration may potentiate the effects already induced by maternal food energy deficiency. Late resorptions may occur to a lesser extent if TTO is administered via food which is the most relevant route for humans.

10.10.6 Comparison with the CLP criteria

Category 1A: *Known human reproductive toxicant*. The classification of a substance in Category 1A is largely based on evidence from humans.

Category 1B: *Presumed human reproductive toxicant*. The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide **clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects**, or if occurring together with other toxic effects the adverse effect on reproduction is considered **not to be a secondary non-specific consequence of other toxic effects**. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

Category 2: *Suspected human reproductive toxicant*. Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects **shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the**

adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

There is no data on humans to inform on the developmental toxicity of Tea Tree Oil, and so classification in category 1A is not appropriate.

Since there is no clear evidence of an adverse effect in the absence of other toxic effects, no classification in category 1B or category 2 for toxicity on development is warranted.

The effects observed in both developmental studies do not indicate that TTO developmental toxicity in rats and rabbits meets classification criteria for this health hazard. Main developmental parameters were not affected therefore they are not considered as significant adverse effects warranting classification for developmental toxicity.

10.10.7 Adverse effects on or via lactation

The potential of Tea Tree Oil to elicit adverse effects on or via lactation has been investigated in a two-generation study in rats (see also section 10.10.1).

Table 53: Summary table of animal studies on effects on or via lactation

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	NOEL/NOAEL [mg/kg bw/day]	Results	Reliability score	Reference
Two generation study in the rat OECD 416 GLP	Tea Tree Oil Purity: 10.30 % α-Terpinene, 20.90 % γ-Terpinene, 1.53 % p-cymene and 42.36 % Terpinen-4-ol (in compliance with ISO pecification) Administration: Oral (gavage) Dose levels: Generation-P: 0, 10, 25 and 50 mg/kg day. Generation-F1: 0, 10, 25 and 38 mg/kg day Treatment related alterations were observed in the reproductive performance at 50 mg/kg bw/day (P generation). Hence, the high dose of 50 mg/kg bw/day was reduced to 38 mg/kg bw/day for the pups selected for F1 generation.	Reproduction/ offspring NOAEL: 25 mg/kg bw/day	<u>38 mg/kg day:</u> ↓pup mean body weight (males + females of F1 generation). ↓Progressive motile sperm (parental F1) <u>50 mg/kg day:</u> ↓No corpora lutea (P) ↓Gestation length (P) ↓Implantations (P) ↓Mean litter size (P) ↓Mean viable litter size (P) ↓Day 4 survival index (P) ↓Male and female fertility indices (P) ↓Sperm motility (P) ↓Cauda epididymal sperm count (P) ↑Percent abnormal sperm (P) *More detailed results are presented in Table 33 - Table 37 (Section 10.10.1)*	1	Anonymous (2017a)

10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

The potential of Tea Tree Oil to elicit adverse effects on or via lactation has been investigated in a two-generation study in rats (see also section 10.10.1).

Study 1: Anonymous (2017a) Tea Tree Oil: Two generation reproduction toxicity study in Wistar rats, OECD 416, GLP

Reliability statement: The study is conducted in accordance with OECD 416. It meets the validity requirements as set out in the test guideline, shows only minor deviations and the test substance reflects the ISO 4730:2004. Hence, the study is considered reliable without restictions (reliability score: 1).Within the study, post-natal survival of pups was not affected by Tea Tree Oil exposure: there were no effects on lactation or viability indices in either generation. The only toxic effect on pups which might be due to substance transfer via milk was seen on bodyweight of the pups of the F1 generation (i.e. F2 litter). During lactation, treatment with Tea Tree Oil significantly reduced mean body weights on Days 1 and 4 in male pups and on Days 1, 4 and 7 in female pups and combined sex at 38 mg/kg bw/day, which is the highest dose tested within this generation. The bodyweights of the P generation pups (i.e. F1 litter) were however not affected even though the dams received 50 mg/kg bw/day. At the end of the lactation period (21 day), body weights recovered and were no longer different from control animals indicating that the body weight reduction, even if treatment related, should not be considered as a severe toxic effect. There was no indication of impaired nursing behavior.

The results of the study do not indicate any direct, adverse effect on the offspring due to transfer of the chemical via the milk or to the quality of the milk.

10.10.9 Comparison with the CLP criteria

According to the Regulation (EC) No 1272/2008 (CLP) Table 3.7.1(b) a substance should be classified for lactation effects when the following applies:

"(a) human evidence indicating a hazard to babies during the lactation period; and/or

(b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or

(c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk."

No data is available to address criteria (a) and (c). The reduced body weight gain of F1 pups during the initial days of lactation is not considered to "*provide clear evidence of adverse effect in the offspring due to transfer in the milk*".

No classification for reproductive toxicity concerning effects on or via lactation is proposed.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

Adverse effects on sexual function and fertility

Based on all provided data Tea Tree Oil TTO warrants classification to subcategory Repr. 2 with hazard statement H361f - Suspected of damaging fertility.

Adverse effects on development

No classification is proposedData conclusive but not sufficient for classification.

Adverse effects on or via lactation

No classification is proposed. Data conclusive but not sufficient for classification.

10.11 Specific target organ toxicity-single exposure

The acute studies that are relevant for the assessment of the specific target organ toxicity of Tea Tree Oil after single exposure are reported in sections 10.1 to 10.3.

Table 54: Summary table of animal studies on STOT SE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reliability score	Reference
Acute oral toxicity study in rats OECD 425 (2008) Acute Oral Toxicity Up-and-Down Procedure GLP Rat, Wistar, Hsd Han: females, 3/group	Tea Tree Oil Purity: 9.7% α-Terpinene, 1.5% p-Cymene, 2.6% 1.8-Cineol, 17.8% γ-Terpinene and 41.5% Terpinen-4-ol (in compliance with ISO specification) Administration: oral, single dose Dose levels: 550 mg TTO/kg (Group 1) and 2000 mg TTO/kg (Group 2) Observation period 14 days	LD_{50} 1049 mg/kg bw <u>Clinical signs:</u> 550 mg TTO/kg: no clinical signs or mortality 2000 mg TTO/kg: Hypoactivity, slight tremors recumbeny, death on day 1 – 2 after dosing.	1	Anonymous (2015)
Acute oral toxicity of Tea Tree Oil in the rat OECD 401 Rat, Sprague Dawley SPF rats - Specific Pathogen-free and non-SPF-rats males, females, 5/group GLP not stated	Tea Tree Oil Purity: not stated Administration: oral, single dose Dose levels: 3, 2.75, 2.6 and 2.5 mL/kg bw (SPF rats – Specific Pathogen-Free) and 2.4, 2.25, 2.15, 2.10 and 1.70 mL/kg bw (non-SPF rats) Observation period 14 days	LD_{50} 2.6 mL/kg bw in SPF rats 1.9 mL/kg bw (≈ 1682 - 1721 mg/kg bw) in non-SPF rats <u>Clinical signs:</u> Surviving SPF and non-SPF rats: lack of tonus in the forelimbs, weeping eyes, bloodied noses.	2	Anonymous 1989a and ECHA dissemination site[34]
Tea Tree Oil: Acute dermal toxicity study in Wistar rats OECD 402 (1987) GLP Rats, Wistar male, female, 2 groups, 5/sex	Tea Tree Oil Purity: 9.7% α-Terpinene, 1.5% p-Cymene, 2.6% 1.8-Cineol, 17.8% γ-Terpinene and 41.5% Terpinen-4-ol (in compliance with ISO spiecification) Administration: dermal, Undiluted test item, Dose level: 2000 mg/kg bw (2.24 ml/kg bw) Exposure duration: 24 hours	LD_{50} > 2000 mg/kg bw	1	Anonymous (2015b)

[34] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/3/2

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reliability score	Reference
Acute dermal toxicity limit test of Tea Tree Oil batch 88/375 in the rabbit OECD 402 Rabbit, New Zealand White 5 males and 5 females	Tea Tree Oil Undiluted test item, 2000 mg/kg bw 24 hours	LD_{50} > 2000 mg/kg bw Clinical signs: slight diarrhoea in 1/10 animals on day 3	2	Anonymous 1989b and ECHA dissemination site[35]
Tea Tree Oil: Acute Inhalation Toxicity Study in Wistar Rats OECD 403 (2009) GLP Rats, Wistar HsdCpb: WU males, females, 5 per group	Tea Tree Oil Purity: 9.45 % α-Terpinene, 5.67 % 1,8-Cineole, 21.04 % γ-Terpinene, 2.35 % p-cymene and 37.98 % Terpinen-4-ol (in compliance with ISO specification) Administration: aerosol Dose levels: 30%, 50% and 70% w/v aerosol of the test item (0.77 ± 0.10 mg/L, 3.69 ± 0.41 mg/L and 5.06 ± 1.13 mg/L air) Exposure duration: Continuous exposition for 4 hours	The acute inhalation LC_{50}, 4h value of Tea Tree Oil in Wistar rats was established to be 3.64 mg/L of air for both male and female rats. Clinical signs: Wet fur was recorded both during and for several hours after exposure, whilst fur staining on the head was recorded on removal from restraint and persisted for several days. In addition, fur staining by the test item was detected in all groups during exposure	1	Anonymous (2010a)

10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

Two acute oral studies were available to assess the specific target organ toxicity of Tea Tree Oil upon single exposure.

Study 1, Anonymous (2015a), Tea Tree Oil: Acute oral toxicity study (Up-and-Down Procedure) in Wistar rats, OECD 425, GLP.

In an acute oral toxicity study from 2015 (see section 10.1), no mortality and clinical signs were observed in all the animals dosed with 550 mg TTO/kg bw (Group 1) throughout the entire 14-day observation period.

The rats dosed with 2000 mg TTO/kg bw (Group 2) exhibited clinical signs such as hypoactivity and slight tremors and died on day 1 or day 2.

All the surviving rats gained weight during the 14-day observation period. The pre-terminal dead rats lost weight when compared to their initial body weight. No abnormalities were observed at necropsy.

[35] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/3/4

Study 2, Anonymous (ECHA dissemination site), Acute oral toxicity of Tea Tree Oil in the rat, OECD 401.

In the second oral toxicity study according to OECD guideline 401, the LD_{50} of TTO was determined in two environmentally derived types of Sprague-Dawley rat. There was a difference in toxicity observed between the two sources of rat. The LD_{50} was found to be 2.6 mL/kg bw in SPF rats and 1.9 mL/kg bw (≈ 1682 – 1721 mg/kg bw) in non-SPF rats, respectively. In rats with different environmental status (i.e. SPF and non-SPF, respectively), no sex-specific difference in the sensitivity towards the test material was observable. The major reaction caused by TTO was complete lack of muscular tone in forelimbs. The surviving animals, however, recovered after few days.

Study 1, Anonymous, (2015b) , Tea Tree Oil: Acute dermal toxicity study in Wistar rats, OECD 402 (1987), GLP.

In an acute dermal toxicity study (see section 10.2), no clinical signs and mortality were observed after application of the undiluted test item at dose of 2000 mg/kg bw (2.24 mL/Kg bw). All rats gained weight during the experimental period. No abnormalities were detected at the necropsy.

Study 2, Anonymous, (ECHA dissemination site), Acute dermal toxicity limit test of Tea Tree Oil batch 88/375 in the rabbit, OECD 402.

In the second dermal toxicity test in rabbits from 1989, no mortality was observed and there were no other signs of toxicity or abnormal behaviour. No significant loss of weight was observed during the observations period.

Study 3, Anonymous, (2010a), Tea Tree Oil: Acute Inhalation Toxicity Study in Wistar Rats, OECD 403 (1987), GLP.

In an acute inhalation toxicity study (see section 10.3), the acute inhalation LC_{50}, 4h value of Tea Tree Oil in Wistar rats was determined to be 3.64 mg/L of air for both male and female rats.

In the control group G1, no toxic signs were recorded throughout the observation period. Toxic signs such as nasal discharge, slight salivation, lethargy, tremors, ataxia, dyspnea, perineum wet with urine, dullness and recumbency were observed in the treated rats (G2, G3 and G4).

The body weights of all surviving animals in the control and the test item groups were increased throughout the observation period. Body weight loss was noted for all dead animals in the test item groups (G2, G3 and G4).

Lung congestion was observed at necropsy in one pre-terminally dead rat of the treated group G4. No abnormalities were found at macroscopic *post mortem* examination of the other animals.

No human data are available

10.11.2 Comparison with the CLP criteria

According to the Regulation (EC) No 1272/2008 (CLP), specific target organ toxicity (single exposure) categories 1 and 2 is defined as specific, non-lethal target organ toxicity arising from a single exposure to a

substance or mixture, which are not covered by the other hazard classes. Category 3 covers transient effects, occurring after single exposure, specifically respiratory tract irritation (RTI) and narcotic effects (NE).

Categories 1 and 2

In the acute toxicity studies performed, no systemic effects were noted after oral and dermal administration. There were no significant non-lethal toxic effects observable that would warrant a classification for specific target organ, single exposure.

After inhalation exposure, clinical signs such as nasal discharge, slight salivation, lethargy, tremors, ataxia, dyspnea, perineum wet with urine, dullness and recumbency were observed in the treated rats at doses of 3.69 mg/L (close to the LC_{50} value).

According to the CLP Guidance, care should be taken not to give a "double classification" for the same effect and as these effects occurred close to the lethal doses they are considered to have been unspecific effects of acute toxicity and are therefore not considered to justify classification in STOT SE.

Category 3

This evaluation is usually based primarily on human data. No human data is available for Tea Tree Oil. However, appropriate animal data, e.g. clinical signs or histopathology data from acute inhalation studies can also be used if available.

According to the CLP Guidance section 3.8.2.2, clinical signs (e.g. dyspnoea, rhinitis etc) and histopathology (hyperaemia, oedema, minimal inflammation, thickened mucous layer) observed in inhalation toxicity studies may justify classification for RTI and lethargy, lack of coordination, loss of righting reflex and ataxia observed in animal studies may justify classification for NE.

Dyspnoea was observed in the tested animals but was closely related to the acute toxicity caused by inhalation and is therefore covered by the classification of Tea Tree Oil as hazard category 4 when regarding inhalation exposure.

10.11.3 Conclusion on classification and labelling for STOT SE

Tea Tree Oil does not need to be classified for STOT SE. Data conclusive but not sufficient for classification.

10.12 Specific target organ toxicity-repeated exposure

The specific target-organ toxicity of Tea Tree Oil upon short-term repeated exposure has been investigated in 28-day and two 90-day studies in rats, and a 90-day study in dogs. A 28-day dermal toxicity study in rabbits is also available. Chronic/carcinogenicity studies in rats and mice are not available. Reproductive and developmental toxicity studies were also considered for STOT RE evaluation.

Table 55: Summary table of animal studies on STOT RE

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Relia-bility score	Reference
28 day oral gavage rat (Sprague-Dawley) 5 m and 5 f/group (40 in	Melaleuca alternifolia, ext., Purity: 100%	5 mg/kg bw/day (actual dose received) 15 mg/kg	No effects observed. NOAEL: 45 mg/kg bw/day (actual dose received)	1	ECHA dissemination site (2017b)[36]

[36] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/20921/7/6/2

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Relia-bility score	Reference
total). OECD 407 EPA OPPTS 870.3050 GLP	Compliant with ISO 4730:2017	bw/day (actual dose received) 45 mg/kg bw/day (actual dose received) Vehicle: corn oil Exposure: At least 28 days (7 days/week).	(male/female) based on: (test mat.)		
28-days, feeding, rats (Wistar rats) Non-GLP	Tea Tree Oil Purity: 9.45 % α-Terpinene, 5.67 % 1,8-Cineole, 21.04 % γ-Terpinene, 2.35 % p-cymene and 37.98 % Terpinen-4-ol (in clompliance with ISO specification) Vehicle: Groundnut oil Administration: gavage Dose levels: 0, 62.5, 125, 250 mg/kg bw/day	NOEL = 62.5 mg/kg body weight/day	<u>At 125 mg/kg bw/day</u> • Degenerative changes in testes • Oligospermia • Epididymal cell debris • Pale liver • Hepatocyte vacuolation • ↑Liver weight <u>At 250 mg/kg bw/day</u> ↓ absolute and relative weights of testes and epididymides • Small sized epididymides and testes • Degenerative changes in testes • Aspermia • Pale liver • Hepatocyte vacuolation • Zona fasciculata hypertrophy (adrenals) • ↑Liver weight • ↑Adrenal weight *More detailed information in presented in Table 56*	2	Anonymous (2010b) N896

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Relia-bility score	Reference
90-days, feeding, rats (Wistar rats – HsdCpb) OECD 408 GLP	Tea Tree Oil Purity: 9.45 % α-Terpinene, 5.67 % 1,8-Cineole, 21.04 % γ-Terpinene, 2.35 % p-cymene and 37.98 % Terpinen-4-ol (in compliance with ISO specification) Vehicle: Groundnut oil Administration: gavage Dose levels: 0, 30, 60, 120 mg/kg bw/day	Males: NOAEL (90 days) = 30 mg/kg bw/day Females: NOAEL (91 days) = 60 mg/kg bw/day	<u>At 60 mg/kg bw/day</u> ↓ Sperm counts and motility ↑ Percent abnormal sperms <u>At 120 mg/kg bw/day</u> ↓ Sperm counts and motility ↑ Percent abnormal sperms ↓ absolute and relative weights of testes and epididymides -degenerative changes in seminiferous tubules -cell debris in tubular lumen of testes and atrophic appearance -sertoli cell vacuolation -sperm granuloma -cell debris in epidydimal duct lumen • Spleen vacuolation (minimal degree) • Tubular dilatation in kidneys (minimal degree) *More detailed information in presented in Table 57*	1	Anonymous (2011b) G7153
90-days, feeding, rats (Wistar rat - Hsd Han) OECD 408 GLP	Tea Tree Oil Purity: 10.3% α-Terpinene, 20.9% γ-Terpinene, 1.36% 1,8-Cineole, 1.53% p-Cymene and 42.36% Terpinen-4-ol. (in compliance with ISO specification) Vehicle: Groundnut oil Administration: gavage Dose levels: 0, 60 mg/kg bw/day	Not estimated.	<u>At 60 mg/kg bw/day</u> ↓ Sperm counts and motility ↑ Percent abnormal sperms - Sperm granuloma - Oligospermia, - Single cell necrosis, - Luminal cell debris - Degeneration/atrophy of seminiferous tubules *More detailed information is presented in Table 58*	1	Anonymous (2016a) G11089

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Relia-bility score	Reference
90-days oral, dogs (Beagle) OECD 409 GLP	Tea Tree Oil Purity: 9.95% α-Terpinene, 20.35% γ-Terpinene, 4.42% 1,8-Cineole, 1.85% p-Cymene and 41.92% Terpinen-4-ol. (in compliance with ISO specification) Vehicle: Sesame oil Administration: gavage Dose rates: 0, 30, 75/60, 180/20 mg/kg bw/day (dose reduction from test day 27 on due to signs of intoxication)	NOAEL (90 days) = 30 mg/kg bw/day	<u>At 75/60 mg/kg bw/day</u> ↓ viability and motility of the canine spermatids <u>At 180/120 mg/kg bw/day</u> ↓ viability and motility of the canine spermatids • Clinical signs (starting within 5 minutes after administration and lasting up to 20 minutes) • ↓Body weight/gain (180mg) • ↓Food consumption (180mg) *More detailed information is presented in Table 59 - Table 61*	1	Anonymous (2018a) 34433
Two generation study in the rat OECD 416 Oral (gavage) GLP	Generation-P: 0, 10, 25 and 50 mg/kg day. Generation-F1: 0, 10, 25 and 38 mg/kg day	Reproduction/ offspring NOAEL: 25 mg/kg bw/day	<u>38 mg/kg day:</u> ↓pup mean body weight (males + females of F1 generation). ↓Progressive motile sperm (parental F1) <u>50 mg/kg day:</u> ↓No corpora lutea (P) ↓Gestation length (P) ↓Implantations (P) ↓Mean litter size (P) ↓Mean viable litter size (P) ↓Day 4 survival index (P) ↓Male and female fertility indices (P) ↓Sperm motility (P) ↓Cauda epididymal sperm count (P) ↑Percent abnormal sperm (P) ↓Body weight/gain (M) ↓Food consumption (M+F) *More detailed results are presented in Table 33 - Table 37 (Section 10.10.1)*	1	Anonymous (2017b)
Prenatal Developmental Toxicity Study in	Tea Tree Oil Purity: 8.18 % α-Terpinene, 1.80 % 1,8-Cineole,	NOAEL, maternal toxicity: 30	<u>60 mg/kg day:</u> ↓Maternal body weight ↓Maternal food intake	1	Anonymous (2012a)

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Relia-bility score	Reference
the rat Oral (gavage) OECD 414 GLP Wistar rats – HsdHan Females	14.23 % γ-Terpinene, 3.86 % p-cymene and 41.73 % Terpinen-4-ol (in compliance with ISO specification) Vehicle: refined peanut oil Administration: gavage Dose rates: 0, 75, 150 and 300 mg/kg/day and 0, 30, 60 and 120 mg/kg/day.	mg/kg/day NOAEL, fetal toxicity: 60 mg/kg/day	<u>120 mg/kg day:</u> ↓Maternal body weight ↓Maternal food intake ↓Fetal weight <u>150 mg/kg day:</u> Clinical signs Incidence of mortality ↓Maternal body weight ↓Maternal food intake <u>300 mg/kg day:</u> Clinical signs Incidence of mortality ↓Maternal body weight ↓Maternal food intake ↑Resorptions *More detailed results are presented in Table 49 - 50 (Section 10.10.5)*		
Prenatal Developmental Toxicity Study in the rabbit Oral (gavage) OECD 414 GLP New Zealand white rabbits 24/group	Tea Tree Oil Purity: 9.95% α-Terpinene, 20.35% γ-Terpinene, 4.42% 1,8-Cineole, 1.85% p-Cymene and 41.92% Terpinen-4-ol. (in compliance with ISO specification) Vehicle: refined peanut oil Administration: gavage Dose rates: 0, 15, 30, and 75 mg/kg/day	NOAEL, maternal toxicity: 75 mg/kg/day NOAEL, fetal toxicity: 30 mg/kg/day NOAEL teratogenicity: 75 mg/kg/day	<u>75 mg/kg day:</u> ↑Post implantation loss *More detailed results are presented in Table 51 - Table 52 (Section 10.10.5)*	1	Anonymous (2018b)

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

Study 1, Anonymous (2010b), Tea Tree Oil: 28-Day Repeated Dose Toxicity Study in Wistar Rats, Non-GLP.

Reliability statement: The study is conducted in accordance with OECD 407. It meets the validity requirements as set out in the test guideline, shows only minor deviations and the test substance reflects the ISO 4730:2004. Howwever, not all required organs have been fixed for histopathological examination and the study reports no information as to GLP. Hence, it is considered reliable with restictions (reliability score: 2).

28 days after oral application at doses of 62.5, 125, and 250 mg/kg bw/day, at the highest dose, decreased weights of testes and epididymides, degenerative changes in both organs and aspermia were observed. At 125 mg/kg bw/day, again degenerative changes in tested and epididymides and oligospermia were observed. Liver and adrenals were affected at the highest dose.

90 days after oral application at doses of 30, 60 and 120 mg/kg bw/day to rats, in males testes and epididymides were affected. At 120 mg/kg bw /day degenerative changes in testes and in epididymides became apparent and remain present after a recovery period of 28 days. At 60 and 120 mg/kg bw/day sperm number, motility and morphology were affected with a trend of recovery after 28 days. Spleen and kidneys were minimally affected.

Table 56: Clinical observations in *Wistar rats* during a 28-day repeated dose toxicity study with TTO

Parameter	**Concentration [mg/kg Bwt/day]**							
	Males				**Females**			
	0	**62.5**	**125**	**250**	**0**	**62.5**	**125**	**250**
No. of Animals per Concentration	6	6	6	6	6	6	6	6
Gross pathology								
Epididymides – small sized	0	0	0	2	NA	NA	NA	NA
Testes – small sized	0	0	0	2	NA	NA	NA	NA
Liver – pale, diffuse	0	0	4	1	0	0	0	2
Histopathology								
Adrenals- hypertrophy-zona fasciculata	0	0	0	0	0	0	0	0
minimal	---	---	---	3	---	---	---	2
Testes								
- degenerative changes-bilateral	0	0	4	6	NA	NA	NA	NA
minimal	---	---	4	3	NA	NA	NA	NA
mild	---	---	---	3	NA	NA	NA	NA
Epididymides - aspermia	0	0	0	5	NA	NA	NA	NA
- oligospermia	0	0	2	1	NA	NA	NA	NA
- cell debris in lumen	0	0	4	0	NA	NA	NA	NA
Liver -hepatocyte vacuolation	0	1	2	6	1	0	1	5
minimal	---	1	2	4	1	---	1	5
mild	---	---	---	2	---	---	---	---
Organ weights								
Epididymides - absolute	-	-	-	↓(34)	NA	NA	NA	NA
- relative	-	-	-	↓(28)	NA	NA	NA	NA
Testes - absolute	-	-	-	↓(43)	NA	NA	NA	NA
- relative	-	-	-	↓(39)	NA	NA	NA	NA
Liver - absolute	-	-	-	-	-	-	↑(19)	↑(23)
- relative	-	-	-	↑(14)	-	-	↑(18)	↑(32)
Adrenals - relative	-	-	-	↑(18	-	-	-	↑(18)

↑ : Statistically significant increase; ↓ : Statistically significant decrease; -: no statistical significance

Study 2, Anonymous, (ECHA dissemination site), Tea Tree Oil: 90-day repeated dose toxicity study in Wistar Rats, OECD 408, GLP.

Reliability statement: The study is conducted in accordance with the former version of OECD 408. It meets the validity requirements as set out in the test guideline, shows only minor deviations and the test substance reflects the ISO 4730:2004. OECD 408 has been updated to place additional emphasize on measurement of endocrine endpoints, such as T3, T4, TSH, LDL, HDL. Since these requirements were not in place at the time of study conduction, the endpoints have not been measured. However, this does not impair the overall reliability and regulatory suitability of the study. Hence, the study is nevertheless considered reliable without restictions (reliability score: 1).

Table 57: Clinical observations in Wistar rats during a 90-day repeated dose toxicity study with TTO

Inlife phase	**Treatment Period**				**Recovery Period**			
Concentration [mg/kg Bwt/day]	0	**30**	**60**	**120**	**0**	**120**	**0**	**120**
No. of Animals per Concentration	10	10	10	10	10	10	10	10
Sacrified on day	91				15		29	
Sperm evaluation (Males)								
Progressive motile sperms %	60.1	---	43.1 ↓* (28)	3.8 ↓ (94)	60.8	7.6 ↓ (88)	63.0	10.2 ↓ (84)
Motile sperms %	84.2	---	64.6 ↓* (23)	16.5 ↓ (80)	82.6	30.8 ↓ (63)	83.0	41.0 ↓ (51)
Sperm morphology (Males)								
Normal sperms	98.9	---	78.5 ↓ (21)	3.8 ↓ (96)	99.4	15.8 ↓ (84)	97.8	22.3 ↓ (77)
Abnormal sperms	106	---	21.5 ↑*	86.18 ↑	0.60	84.2 ↑	2.20	77.7 ↑
Sperm counts (Males)								
Cauda epididymis wt	0.247	---	---	---	0.255	---	0.258	0.199 ↓ (23)
No. of sperms per cauda epididymis	190.03	---	---	---	205.40	---	187.15	63.40 ↓ (66)
No. of sperms per gram of cauda epididymis	770.88	---	604.59 ↓ (22)	457.31 ↓ (41)	803.51	353.59 ↓ (56)	727.65	310.61 ↓ (57)
Gross pathology (Males)								
Epididymides – Abscess	0	0	0	6	0	4	0	1
Testes – small and flacid	0	0	0	1	0	0	0	4
- flaccid	0	0	0	3	0	0	0	0
- small	0	0	0	0	0	2	0	4
Histopathology (Males)								
Testes No. examined	10	10	10	10	5	10	5	10
Degenerative changes – seminiferous tubules	0	0	0	8	0	9	0	8
minimal	0	0	0	4	0	3	0	2
mild	0	0	0	2	0	4	0	4
moderate	0	0	0	1	0	2	0	2
marked	0	0	0	1	0	0	0	0
Sertoli cell vacuolation	0	0	0	9	0	10	0	9
minimal	0	0	0	7	0	6	0	5
mild	0	0	0	2	0	4	0	4
Sperm stasis	0	0	0	0	0	4	0	0
minimal	0	0	0	0	0	4	0	0
Epididymides No. examined	10	10	10	10	5	10	5	10
Sperm granuloma	0	0	0	4	0	6	0	1
Cell debris in lumen	0	0	1	7	0	9	0	9
minimal	0	0	1	6	0	1	0	2
mild	0	0	0	0	0	6	0	5
moderate	0	0	0	0	0	1	0	2
marked	0	0	0	0	0	1	0	0
Oligospermia	0	0	0	3	0	5	0	6
Aspermia	0	0	0	1	0	0	0	0
Kidneys No. examined	10	10	10	10	5	10	5	10
Dilatation of tubules	0	0	0	3	0	0	0	0
minimal	0	0	0	3	0	0	0	0
Spleen No. examined	10	10	10	10	5	10	5	10
Vacuolation	0	0	0	5	0	0	0	0
minimal	0	0	0	5	0	0	0	0
Organ weights (Males and females)								
Epididymides (males)								
- absolute	1.497	---	---	---	1.561	---	1.521	1.154 ↓ (24)
- ratios to Bwt	0.382	---	---	---	0.400	---	0.390	0.290 ↓ (26)
Testes (males)								
- absolute	3.787	---	---	---	3.808	---	3.760	2.855 ↓ (24)
- ratios to Bwt	0.970	---	---	---	0.974	---	0.967	0.717

Inlife phase	Treatment Period				Recovery Period			
Concentration [mg/kg Bwt/day]	0	**30**	**60**	**120**	**0**	**120**	**0**	**120**
								↓ (26)
Liver (females) - ratios to Bwt	2.766	---	3.077 ↑ (11)	3.025 ↑ (9)	---	---	---	---

↑ : Statistically significant increase; ↓ : Statistically significant decrease; *: Apparent decrease -: No statistical significance; Values in parenthesis indicate % change

Study 3: Anonymous (2016a) Tea Tree Oil: 90-Day Repeated Dose Toxicity Study in Wistar Rats, OECD 408 (1998)

A second 90-day study especially designed with a prolonged recovery period (Anonymous (23), 2016) was performed in rats only at 60 mg/kg bw/day. Testicular and epididymal findings were comparable to those of the initial 90-day study in rats. All effects recovered after 8 weeks without dosing.

Table 58: Clinical observations in *Wistar rats* during a 90-day repeated dose toxicity study with TTO

Inlife Period		**Treatment Period**		**Recovery Period**					
Day of Sacrifice		**91**		**147 (8 weeks)**		**175 (12 weeks)**		**203 (16 weeks)**	
Concentration (mg/kg bw/day)		**0**	**60**	**0**	**60**	**0**	**60**	**0**	**60**
No. of Animals per Concentration		10	10	10	10	10	10	10	10
Sperm evaluation									
Motility	Progressive motile sperms %	68.20	19.40* ↓(72)	65.30	68.60	54.70	48.50	61.30	59.10
	Motile sperms %	94.10	83.00* ↓(12)	87.30	89.30	76.00	70.60	82.60	81.20
Morphology	Normal sperms	98.00	25.35* ↓(74)	97.20	96.25	86.90	85.39	96.60	95.30
	Abnormal sperms	2.00	74.65* ↑(38folds)	2.80	3.75	13.10	14.61	3.40	4.70
Cauda epididymal sperm counts	Cauda epididymis weight (g)	0.22	0.26	0.24	0.24	0.22	0.25	0.25	0.24
	No. of sperms per cauda epididymis (x 10^6)	198.53	127.88	189.70	181.38	158.03	214.35	214.40	201.05
	No. of sperms per gram of cauda epididymis	902.83	474.35* ↓(850)	799.00	767.03	672.36	785.78	858.89	835.89
Gross Pathology									
EPIDIDYMIDES – Abscess(es); bilateral; tail		0	1	0	0	0	1	0	0
– small; bilateral		0	0	0	0	1	0	0	0
TESTES – Enlarged; unilateral		0	1	0	0	0	0	0	0
– Small/flaccid; bilateral		0	0	0	0	1	2	0	0
– Focus (i); white; bilateral; multiple		0	0	0	0	0	1	0	0
Histopathology									
LEFT EPIDIDYMIS No. examined		(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Sperm granuloma; cauda; focal		0	2	0	0	0	1	0	0
mild		—	1	—	—	—	—	—	—
moderate		—	1	—	—	—	1	—	—
Oligospermia		0	5	0	0	1	2	0	0
minimal		—	5	—	—	—	—	—	—
moderate		—	—	—	—	1	2	—	—
Cellular debris in duct lumen		0	1	0	0	1	2	0	0
minimal		—	1	—	—	—	1	—	—
mild		—	—	—	—	1	1	—	—

Inlife Period	Treatment Period		Recovery Period					
Day of Sacrifice	**91**		**147 (8 weeks)**		**175 (12 weeks)**		**203 (16 weeks)**	
Concentration (mg/kg bw/day)	**0**	**60**	**0**	**60**	**0**	**60**	**0**	**60**
No. of Animals per Concentration	10	10	10	10	10	10	10	10
Single cell necrosis; caput	0	1	0	0	0	0	0	0
minimal	—	1	—	—	—	—	—	—
Chronic active inflammation; cauda	0	1	0	0	0	0	0	0
minimal	—	1	—	—	—	—	—	—
TESTES No.examined	(10)	(10)	(-)	(-)	(1)	(2)	(-)	(-)
Degeneration/atrophy; seminiferous tubule; unilateral	0	1	0	0	0	0	0	0
mild	—	1	—	—	—	—	—	—
Degeneration/atrophy; seminiferous tubule; bilateral	0	0	0	0	1	2	0	0
mild	—	—	—	—	—	1	—	—
moderate	—	—	—	—	1	—	—	—
severe	—	—	—	—	—	1	—	—
Hypertrophy/hyperplasia; interstitial cell; bilateral	0	0	0	0	1	2	0	0
minimal	—	—	—	—	1	2	—	—
Sperm granuloma; bilateral	0	0	0	0	0	2	0	0
minimal	—	—	—	—	—	1	—	—
moderate	—	—	—	—	—	1	—	—

↑ : Statistically significant increase; ↓ : Statistically significant decrease; *: Statistically significant; Values in parenthesis indicate % change

Study 4, Anonymous , (2018a), Repeated dose 90-Day oral toxicity study of Tea Tree Oil in Beagle dogs, OECD 409, GLP.

Reliability statement: The study is conducted in accordance with OECD 409. It meets the validity requirements as set out in the test guideline, shows only minor deviations and the test substance reflects the ISO 4730:2004. Hence, the study is considered reliable without restictions (reliability score: 1).

In a 90-day studies performed in dogs at 0, 30, 75/60, 180/20 mg/kg bw/day, viability and motility of the canine spermatids were decreased at medium and high concentrations. No further signs of toxicity were observed.

Table 59: Changes in sperm viability and motility of Beagle dogs treated with Tea Tree Oil compared to the control group at the end of the treatment period (test week 13)

Parameter / Concentration		Control	75/60 mg/kg	180/120 mg/kg
Sperm Viability %	Alive / Dead	83.4 / 16.6	54.8 / 45.2*	63.1 / 36.9*
Sperm Motility %	Progressive motile sperms	54.5	9.7*	29.4*
	estimated motility	68.8	20.0*	43.0*
	Immotility	29.3	76.0*	55.6*

*: statistically significant at $p \leq 0.01$ (chi^2-test)

Table 60: Statistically significant differences results of the sperm analysis in Beagle dogs treated with Tea Tree Oil compared to the control

Parameter	Increase ↑ Decrease ↓	Concentration / Sex	Test day(s)	Statistical significance	Reason
Weight of Ejaculate	↑	75/60 mg/kg / m	13	$p \leq 0.05$	A
Estimated Motility	↑	30 mg/kg / m	13	$p \leq 0.01$	A
Immotility	↓	30 mg/kg / m	13	$p \leq 0.01$	A

m: male; A: the slight alteration in comparison to control animals is without any biological relevance

Table 61: Food and drinking water consumption during a repeated dose 90-day oral toxicity study of Tea Tree Oil in Beagle dogs

Increase ↑ Decrease ↓	Concentration / Sex	Test week(s)	Statistical significance	Reason
Summary of the food and drinking water consumption during the treatment period				
↑	180/120 mg/kg / m	6 12	$p \leq 0.05$ $p \leq 0.01$	A A
↑	30 mg/kg / f	6 7	$p \leq 0.01$ $p \leq 0.05$	A A
↑	180/120 mg/kg / f	6, 13	$p \leq 0.01$	A
Summary of the food and drinking water consumption during the recovery period				
↑	180/120 mg/kg / f	15 16	$p \leq 0.05$ $p \leq 0.01$	A A

m: male; f: female; A: the slight alteration in comparison to control animals is without any biological relevance

Besides the adverse effects on reproduction described under Point 10.10, within the reproductive and developmental toxicity studies only (in part reversible) reduction in body weight, body weight gain and food consumption were described as further treatment related effects. The post-implantation loss observed in the developmental toxicity study in rabbits (Anonymous (33), 2018b) can be considered as a consequence of maternal toxicity adversely supported by extreme overexposure of TTO caused by gavage administration.

10.12.2 Comparison with the CLP criteria

Regarding all available repeated dose toxicity studies, it becomes clear that Tea Tree Oil has a detrimental effect on spermatogenesis. However, as extensively discussed under Point 10.10., it is most likely that these effects were due to the administration type (gavage vs. dietary). Effects were seen in studies where Tea Tree Oil was administered by gavage. For other terpenes (which were also content of TTO) it was shown that sperm damage does not occur after dietary administration. Gavage administration can be regarded as a non-relevant route of exposure to humans. Furthermore, no exposure of TTO as a plant protection product to humans is expected since there is a no-residue situation of the treated crops. Therefore, no classification is warranted for STOT RE with respect to sperm impairment.

The repeated-dose toxicity of TTO by the oral route has been investigated in a 28-day and two 90-day studies in rats, a 90-day study in dogs, a 3-week dermal toxicity study in rats and by a two-generation toxicity study in rats. There were no long-term/chronic studies available for TTO. Although the developmental studies are also repeated dose toxicity studies, they were not considered for an assessment of STOT RE here. The studies lack histopathological examination of the dams and organ weights and necropsy were also limited. Taken together that extrapolation of effective doses using Haber's law can lead to large uncertainties, especially in

studies with short exposure durations, and the toxicological gain is limited by the study design, it appeared reasonable not to include this study type for STOT RE evaluation. No human data is available.

In the following, effects on the liver after repeated dose administration of TTO are discussed:

In the non-GLP 28-day oral study in rats, liver weights were increased in the highest dose group (250 mg/kg bw/day) in males and at the mid (125 mg/kg bw/day) and high dose group in females. At the highest dose, minimal/mild liver vacuolation was observed histopathologically in both sexes (6 males and 5 females affected) at the highest dose and in males at the mid dose (2 animals affected).

In the 90-day oral study in rats relative liver weights were increased in females at the mid (60 mg/kg bw/day) and the highest (120 mg/kg bw/day) tested dose. There were no corresponding histopathological alterations found. After a recovery period of 8 weeks, liver weights were not different from control animals, indicating an adaptive response during test item application. In males, the liver remained totally unaffected.

A similar picture was observed within the second 90-day study in rats. Minimal liver weight increase at 60 mg/kg bw/day in males was then absent after a recovery period up to 16 weeks.

Within the 90-day study in beagle dogs, there were no liver effects observed in any treatment group which were different from the control animals.

In the parental animals (P and F1) within the two-generation toxicity study, there were minimal weight decreases observed in males and females without dose-dependency and without corresponding alterations in histopathology. The study authors did not consider these weight changes as toxicologically significant.

There is no information of liver effects after dermal application of TTO for 30 days.

Hepatic vacuolation as exclusively observed in the non-GLP 28-day study in rats is often described to be involved in adaptive processes to resist further insults by foreign substances. Also increases in liver weight, which is described in the 90-day and 28-day studies in rats, can be observed during adaptive processes after exposure to xenobiotics. The fact that after a short recovery period no increase in liver weight was observable any longer supports the assumption that both, vacuolation and weight increase are part of an adaptive process rather than an effect of toxicological significance.

As described in the guidance on the application of the CLP criteria[37], more weight should be usually given to studies of a longer duration (28 days or more) because animals may not have fully adapted to the exposure in studies of shorter durations and also because longer duration studies tend to include more thorough and extensive investigations (e.g. in terms of detailed pathology and haematological effects etc) which can generally give more substantial information compared to shorter duration studies. Since the available 28-day study in rats was not performed under GLP conditions, in the present case, the 90-day studies should clearly be given preference.

Taken together, within all available animal studies of sufficient reliability, no life-threatening changes (e.g. necrosis) have been observed in the liver. There were no further indications of functional impairment (e.g. increased serum levels of liver enzymes) after TTO administration. Therefore, the available data does not support a classification for specific target organ toxicity following repeated exposure.

[37] ECHA-17-G-21-EN; 10.2823/124801

10.12.3 Conclusion on classification and labelling for STOT RE

No classification is warranted for STOT RE. Data conclusive but not sufficient for classification.

10.13 Aspiration hazard

Table 63: Summary table of evidence for aspiration hazard

	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
experimental study OECD TG 114 GLP	Melaleuca alternifolia, ext., purity 100%	The viscosity of Tea Tree Oil was determined in triplicate at 20°C and 40°C using a reverse flow viscometer.	Kinematic viscosity of tea tree oil: 2.86 mm2/s at 20°C 1.71 mm2/s at 40°C Dynamic viscosity: 2.54 mPa/s at 20°C 1.52 mPa/s at 40°C	Comb (2007) (REACH registration dossier,)[38]

Tea Tree Oil is a mixture of several components (please refer to point 1.1), that can be attributed to different chemical subgroups.

According to information provided on ECHA website several components of TTO have been notified with classification and labelling for aspiration hazard according to Regulation (EC) No. 1272/2008 by different notifiers. The content of these components in Tea Tree Oil may vary between 19 and 68% (see Table 64).

Table 64: Tea Tree Oil components: occurrence in the extract (acc. to ISO 4730:2004) and allocation to chemical groups (hydrocarbons as defined above are underlined) with data on theirs classification under Regulation (EC) No. 1272/2008 (acc. to substance information on ECHA website: https://echa.europa.eu)

	Name	Min. %	Max. %	C&L as Asp. Tox. 1, H304
Monocyclic monoterpenes, Aliphatic and aromatic hydrocarbons	**γ-Terpinene**	**10**	**28**	Notified classification and labelling according to CLP criteria (Asp. Tox. 1 in >90% notifications)
	α-Terpinene	**5**	**13**	Classified as Asp. Tox. 1 according to RAC opinion proposing harmonised classification and labelling of alpha-terpinene (CAS No: 99-86-5) No: CLH-O-0000001412-86-274/F (Adopted 15 March 2019)
	α-Terpinolene	**1.5**	**5**	Notified classification and labelling according to CLP criteria (Asp. Tox. 1 in 100% notifications)
	Limonene	**0.5**	**1.5**	Notified classification and labelling according to CLP criteria (Asp. Tox. 1 in 50% notifications)

[38] https://echa.europa.eu/de/registration-dossier/-/registered-dossier/20921/4/23

	Name	Min. %	Max. %	C&L as Asp. Tox. 1, H304
	p-Cymene	**0.5**	**8**	Classified as Asp. Tox. 1 according to RAC opinion proposing harmonised classification and labelling of p-cymene EC Number: 202-796-7 (CAS No: 99-87-6) No: CLH-O-0000001412-86-273/F (Adopted 15 March 2019)
Monocyclic monoterpenes, aromatic unsaturated tertiary alcohols	Terpinen-4-ol	30	48	Not classified
	α-Terpineol	1.5	8	Not classified
Bicyclic monoterpenes	1,8-Cineole (Eucalyptol)	trace	15	Not classified
	α-Pinene	**1**	**6**	Notified classification and labelling according to CLP criteria (Asp. Tox. 1 in >75% notifications)
	Sabinene	**trace**	**3.5**	Notified classification and labelling according to CLP criteria (Asp. Tox. 1 in >60% notifications)
Polycyclic sesquiterpenes, Cadinane group	δ-Cadinene	trace	3	Not classified
Polycyclic sesquiterpenes Aromadendrene group	**Aromadendrene**	**0.5**	**3**	Notified classification and labelling according to CLP criteria (Asp. Tox. 1 in 100% notifications)
	Ledene (Viridiflorene)	trace	3	Not classified
Polycyclic sesquiterpenes, Aromadendrene group, Alcohols	Globulol	trace	1	Not classified
	Viridiflorol	trace	1	Not classified

10.13.1Short summary and overall relevance of the provided information on aspiration hazard

Based on information on aspiration hazard of Tea Tree Oil and its constituents TTO should be classified in Category 1 for aspiration toxicity in accordance with the Regulation (EC) No 1272/2008.

10.13.2 Comparison with the CLP criteria

Under Regulation (EC) No. 1272/2008: "where the mixture itself has not been tested to determine its aspiration toxicity, but there are sufficient data on the individual ingredients and similar tested mixtures to adequately characterise the hazard of the mixture, these data shall be used in accordance with the bridging principles set out in section 1.1.3. of Annex I to CLP regulation. However, in the case of application of the dilution bridging principle, the concentration of aspiration toxicant(s) shall be 10% or more". Taking into account that concentration of aspiration toxicants in Tea Tree Oil is higher than 10% (19 - 68%) the TTO should be classified for aspiration toxicity, too.

In the TTO REACH registration dossier (https://echa.europa.eu/)[39] kinematic viscosity of tea tree oil (containing >10% hydrocarbons) is 1.71 mm^2/s at 40°C (Comb (2007). Acording to Regulation (EC) 1272/2008

[39] https://echa.europa.eu/de/registration-dossier/-/registered-dossier/20921/4/23

mixture which contains a total of 10 % or more of a substance or substances classified in Category 1, and has a kinematic viscosity of 20,5 mm 2 /s or less, measured at 40 o C, shall be classified as Asp. Tox. 1, H304(May.

10.13.3 Conclusion on classification and labelling for aspiration hazard

The classification for Aspiration Hazard Cat. 1 (Asp. Tox. 1, H304: May be fatal if swallowed and enters airways) for Tea Tree Oil is warranted.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

For details of the summarized studies on environmental hazards of Tea Tree Oil, please refer to Annexes 3 and 4 of the CLH report.

11.1 Rapid degradability of organic substances

Table 65: Summary of relevant information on rapid degradability

<table>
<tr><th>Study</th><th>Method / Results</th><th>Remarks</th><th>Relia - bility score</th><th>Reference</th></tr>
<tr><td>Tea Tree Oil (TTO): ready biodegradability – CO_2 in sealed vessels (Headspace Test)
OECD 310</td><td>Tea Tree Oil:
Purity: α-Terpinene: 10.433 % (w/w), 1,8-Cineole: 3.277 % (w/w), γ-Terpinene 21.667 % (w/w), p-Cymene 2.277 % (w/w), Terpinene-4-ol 40.067 % (w/w)
The ready biodegradability of Tea Tree Oil was determined with a non-adapted sludge over a test period of 28 days in the Headspace Test according to OECD 310.</td><td>In the toxicity control treatments (test medium + reference item + TTO), the test substance TTO did not have any toxic effect on the microorganisms.</td><td>1</td><td>Anonymous (2010c)</td></tr>
<tr><td colspan="5">Aerobic soil degradation</td></tr>
<tr><td>[^{14}C]Terpinene-4-ol and gamma-Terpinene, p-Cymene, 1,8-Cineole, Globulol /
Viridiflorol and Aromadendrene from Tea Tree Oil
Aerobic Degradation in one Soil at 20 °C in the Dark
OECD 307, GLP</td><td>The route of [^{14}C]Terpinene-4-ol and rate of degradation of [^{14}C]Terpinene-4-ol, gamma-Terpinene, p Cymene, 1,8-Cineole, (-)-Globulol/Viridiflorol and (+)-Aromadendrene from Tea Tree Oil were investigated under aerobic conditions at 20 °C in the dark using one soil of European origin (2.4). The study was performed with radio-labelled [^{14}C]Terpinene-4-ol and non-radio-labelled gamma-Terpinene, p-Cymene , 1,8-Cineole, ( ) Globulol / Viridiflorol and (+)-Aromadendrene from Tea Tree Oil over a period of 768 hours (32 days).
Results:
<table>
<tr><th>Test Item</th><th>Kinetic Model</th><th>DT_{50} [hours]</th><th>DT_{90} [hours]</th><th>Chi^2 Error [%]</th><th>Visual Assessment *</th></tr>
<tr><td>[^{14}C]Terpinene-4-ol</td><td>SFO</td><td>10</td><td>33.3</td><td>15.9</td><td>+</td></tr>
<tr><td>gamma-Terpinene</td><td>SFO</td><td>0.861</td><td>2.86</td><td>4.12</td><td>+</td></tr>
<tr><td>p-Cymene</td><td>SFO</td><td>0.995</td><td>3.3</td><td>4.97</td><td>+</td></tr>
<tr><td>1,8-Cineole</td><td>SFO</td><td>2.39</td><td>7.95</td><td>9.94</td><td>+</td></tr>
<tr><td>(-)-Globulol</td><td>SFO</td><td>151</td><td>503</td><td>8.91</td><td>+</td></tr>
<tr><td>(+)-Aromadendrene</td><td>SFO</td><td>70.5</td><td>234</td><td>19.7</td><td>+</td></tr>
</table>
* Visual Assessment: + = good, o = moderate, - = poor</td><td>[^{14}C]Terpinene-4-ol degraded to CO_2, non-extractable residues and minor metabolites.</td><td>1</td><td>Anonymous (2018c)</td></tr>
</table>

<table>
<tr><th>Study</th><th>Method / Results</th><th>Remarks</th><th>Relia - bility score</th><th>Reference</th></tr>
<tr><td>gamma-Terpinene, p-Cymene, 1,8-Cineole, Globulol / Viridiflorol and Aromadendrene - Aerobic Degradation in Three Soils at 20 °C in the Dark

OCED 307

GLP</td><td>The rate of degradation of gamma-Terpinene, p-Cymene, 1,8-Cineole, Globulol / Viridiflorol and Aromadendrene was investigated under aerobic conditions at 20 ± 2 °C in the dark using three soils of European origin (LUFA 2.1, LUFA 2.2 and LUFA 6S). The study was performed over a period of 32 days.

Results:
<table>
<tr><th>Test Item</th><th>Soil</th><th>Best Fit Kinetic Model</th><th>DT_{50} [h]</th><th>DT_{90} [h]</th><th>Chi^2 Error [%]</th><th>Visual Assessment *</th></tr>
<tr><td rowspan="3">p-Cymene</td><td>LUFA 2.1</td><td>FOMC</td><td>3.02</td><td>26.8</td><td>4.92</td><td>+</td></tr>
<tr><td>LUFA 2.2</td><td>SFO</td><td>12.8</td><td>42.6</td><td>13.3</td><td>+</td></tr>
<tr><td>LUFA 6S</td><td>SFO</td><td>5.96</td><td>19.8</td><td>9.7</td><td>+</td></tr>
<tr><td rowspan="3">1,8-Cineole</td><td>LUFA 2.1</td><td>SFO</td><td>9.43</td><td>31.3</td><td>8.08</td><td>+</td></tr>
<tr><td>LUFA 2.2</td><td>SFO</td><td>16.3</td><td>54</td><td>14.7</td><td>+</td></tr>
<tr><td>LUFA 6S</td><td>SFO</td><td>7.36</td><td>24.5</td><td>8.76</td><td>+</td></tr>
<tr><td rowspan="3">gamma-Terpinene</td><td>LUFA 2.1</td><td>DFOP</td><td>1.78</td><td>14.5</td><td>2.8</td><td>+</td></tr>
<tr><td>LUFA 2.2</td><td>DFOP</td><td>1.63</td><td>13.4</td><td>0.962</td><td>+</td></tr>
<tr><td>LUFA 6S</td><td>SFO</td><td>3.56</td><td>11.8</td><td>8.94</td><td>+</td></tr>
<tr><td rowspan="3">(+)-Aromadendrene</td><td>LUFA 2.1</td><td>FOMC</td><td>81.9</td><td>361</td><td>8.8</td><td>+</td></tr>
<tr><td>LUFA 2.2</td><td>SFO</td><td>186</td><td>617</td><td>11.5</td><td>o</td></tr>
<tr><td>LUFA 6S</td><td>FOMC</td><td>63.4</td><td>779</td><td>12.1</td><td>+</td></tr>
<tr><td rowspan="3">(-)-Globulol</td><td>LUFA 2.1</td><td>SFO</td><td>262</td><td>871</td><td>11.1</td><td>o</td></tr>
<tr><td>LUFA 2.2</td><td>SFO</td><td>186</td><td>619</td><td>11.7</td><td>o</td></tr>
<tr><td>LUFA 6S</td><td>SFO</td><td>167</td><td>556</td><td>8.01</td><td>o</td></tr>
</table>
* Visual Assessment: + = good, o = moderate, - = poor</td><td>In the aerobic soil degradation study, 10 minor transformation products evolved. None of the metabolites reached 5 % AR. Therefore, no studies on metabolites are required.</td><td>1</td><td>Anonymous (2018d)</td></tr>
<tr><td colspan="5">Adsorption and desorption of the active substance</td></tr>
<tr><td>Estimation of Adsorption-Coefficient on</td><td>The aim of this study was the determination of the adsorption coefficient of Terpinene-4-ol, gamma-Terpinene, p-Cymene, 1,8-Cineole, Globulol /</td><td></td><td>1</td><td>Anonymous (2018e)</td></tr>
</table>

<table>
<tr><th>Study</th><th>Method / Results</th><th>Remarks</th><th>Relia - bility score</th><th>Reference</th></tr>
<tr><td>Soil of Terpinene-4-ol, gamma-Terpinene, p-Cymene, 1,8-Cineole, Globulol / Viridiflorol and Aromadendrene using High Performance Liquid Chromatography (HPLC)

OECD 121

GLP</td><td>Viridiflorol and Aromadendrene on soil and on sewage sludge (K_{OC}) by high-performance liquid chromatography (HPLC) method according to EC method C.19 (440/2008) and OECD guideline 121.

Results:
<table>
<tr><th>Test Item</th><th>Mean retention time of two runs [min]</th><th>Absolute deviation [min]</th><th>Mean log K_{OC} of two runs</th><th>Absolute deviation of log K_{OC}</th></tr>
<tr><td>Terpinene-4-ol</td><td>3.40</td><td>0.01</td><td>1.95</td><td>0.00</td></tr>
<tr><td>gamma-Terpinene</td><td>6.06</td><td>0.03</td><td>3.36</td><td>0.01</td></tr>
<tr><td>p-Cymene</td><td>5.46</td><td>0.02</td><td>3.13</td><td>0.01</td></tr>
<tr><td>1,8-Cineole</td><td>3.19</td><td>0.01</td><td>1.77</td><td>0.00</td></tr>
<tr><td>(-)-Globulol</td><td>5.74</td><td>0.01</td><td>3.24</td><td>0.00</td></tr>
<tr><td>(+)-Aromadendrene</td><td>13.88</td><td>0.08</td><td>> 5.0 (5.05) *</td><td>0.00</td></tr>
</table>
* above the determination limit of the method (5.0 according to OECD 121) but within the calibration range (log K_{OC} of the highest reference standard 4,4'-DDT is 5.63)</td><td></td><td></td><td></td></tr>
<tr><td colspan="5">Fate and Behaviour in Water and Sediment</td></tr>
<tr><td>Hydrolytic degradation

Expert statement</td><td rowspan="2">Due to their high vapour pressure and rather low water solubility, especially of the terpene hydrocarbons, most of the TTO constituents will volatilise from surface water within a very short time period after application. This is indicated by the high Henry constants and vapor pressures of the constituents. As terpenes do not persist in water, no testing of hydrolysis is required. This conclusion is supported by calculations according to the German EVA model.

Estimations of the photochemical oxidative degradation and experimental data show that all TTO constituents rapidly degrade in the gas phases under the influence of light.

Based on their low solubility in water, their high volatility and high reactivity under the influence of light, it may be assumed that the persistence time of the different TTO constituents in flora, soil and surface waters is rather low. Furthermore, based on the fact that the TTO constituents react rapidly with OH, NO_3 radicals and O_3 their residence time in the troposphere is also considered to be short.

Further experimental data on hydrolysis and photolysis of TTO and its components are therefore not deemed necessary.</td><td rowspan="2"></td><td></td><td rowspan="2">Anonymous (2007a) (RAR 2018)</td></tr>
<tr><td>Photolysis

Expert statement</td><td></td></tr>
<tr><td>None, statement</td><td>None of the Components, with the exception of p-Cymene (max. 8% of total), contains conjugated double bonds. Accordingly, none of these substances have</td><td></td><td></td><td></td></tr>
</table>

<table>
<tr><th>Study</th><th>Method / Results</th><th>Remarks</th><th>Relia - bility score</th><th>Reference</th></tr>
<tr><td>Direct photochemical degradation</td><td>significant absorption at 290 nm or above.

Therefore, direct aqueous photolysis is at most relevant only for p-Cymene. However, the spectra for p-Cymene in Roehri, C. (2017) clearly show that p-Cymene does not absorb at wavelength (λ) ≥ 290 nm. Therefore, no studies on direct photochemical degradation are deemed necessary.</td><td></td><td></td><td></td></tr>
<tr><td>[^{14}C]Terpinene-4-ol and gamma-Terpinene, p-Cymene, 1,8-Cineole, Globulol /Viridiflorol and Aromadendrene from Tea Tree Oil

Aerobic Degradation and Metabolism in two Water/Sediment Systems

OECD 308

GLP</td><td>Results:
<table>
<tr><th>W/S system</th><th>Component</th><th>DT$_{50}$ [h]</th><th>DT$_{90}$ [h]</th><th>Chi^2</th></tr>
<tr><td rowspan="6">Pfalz Hanhofen</td><td>Terpinene-4-ol</td><td>196</td><td>652</td><td>7.93</td></tr>
<tr><td>γ-Terpinene</td><td>1.49</td><td>4.95</td><td>2.22</td></tr>
<tr><td>p-Cymene</td><td>1.61</td><td>5.34</td><td>5.6</td></tr>
<tr><td>1,8-Cineole</td><td>50.7</td><td>169</td><td>13.5</td></tr>
<tr><td>(-)-Globulol</td><td>206</td><td>684</td><td>18.1</td></tr>
<tr><td>(+)-Aromadendren</td><td>23.4</td><td>77.7</td><td>23.8</td></tr>
<tr><td rowspan="6">Humsterbach</td><td>Terpinene-4-ol</td><td>104</td><td>345</td><td>6.95</td></tr>
<tr><td>γ-Terpinene</td><td>1.16</td><td>3.87</td><td>8.6</td></tr>
<tr><td>p-Cymene</td><td>1.6</td><td>5.31</td><td>6.81</td></tr>
<tr><td>1,8-Cineole</td><td>15.2</td><td>50.4</td><td>7.36</td></tr>
<tr><td>(-)-Globulol</td><td>84</td><td>279</td><td>12.4</td></tr>
<tr><td>(+)-Aromadendrene</td><td>8.38</td><td>27.8</td><td>12.7</td></tr>
</table></td><td>No metabolites > 10% were observed in either soil or water/sediment study. The rapid metabolism of Tea Tree Oil observed both in soil and water/sediment, and the fact that Tea Tree Oil is readily biodegradable, indicates that Tea Tree Oil components is used as a readily available energy source.</td><td>1</td><td>Anonymous (2018f)</td></tr>
</table>

11.1.1 Ready biodegradability

The rapid degradability of TTO was tested in an OECD 310 GLP study.

Study 1, Anonymous (2010c), Tea Tree Oil (TTO): ready biodegradability – CO_2 in sealed vessels (Headspace Test), OECD 310, GLP.

Reliability statement: The study was conducted in line with OECD 310 and meets the respective validity criteria reported therein. The composition of the test substance reflects the ISO 4730:2004. Moreover, the method is considered suitable according to point 11 in OECD 310, since complete aerobic degradation could be demonstrated although some of the components of the active substance exceed the Henry‘s law constant criterion of maximum 50 (Pa x m³)/mol. The study also includes an analytical method fully validated. No relevant deviations from OECD 310 were identified. Hence, the study is considered reliable (reliability score: 1).

The test item was tested at a nominal dose of 10 mg C/L in triplicates.
The biodegradation was followed by TIC analysis of the produced CO_2 by the respiration of bacteria.

Tea Tree Oil was readily biodegradable under the test conditions. The 10% level was reached after 2 days. The 60% pass level was reached within the 10-d window after 5 days. The maximum biodegradation came to 106% after 28 days. Thus, TTO is readily biodegradable by the terms of this test.
In accordance with the EC Directives on dangerous preparations 1272/2008, substances are considered rapidly degradable in the environment if one of the following criteria holds true:
(a) if, in 28-day ready biodegradation studies, at least the following levels of degradation are achieved:
(i) tests based on dissolved organic carbon: 70 %;
(ii) tests based on oxygen depletion or carbon dioxide generation: 60 % of theoretical maximum.

These levels of biodegradation must be achieved within 10 days of the start of degradation which point is taken as the time when 10 % of the substance has been degraded, unless the substance is identified as an UVCB or as a complex, multi- constituent substance with structurally similar constituents.

It is obvious from the OECD test on Ready Biodegradability that Tea Tree Oil can be regarded as readily biodegradable.
This finding is further supported by Literature data on biodegradation of terpene components (see following table).

Table 66: Literature data on biodegradation of terpene components

Substance	Study/endpoint type	Results	Reliability score	Reference
Ready biodegradability				
Limonene	Unacclimated coniferous forest soil extracts, aerobic conditions at 23°C; Measured: biomass increase and headspace CO_2	Lag period (h): 182 Max. degr. rate (mg/L/h): 0.044 Norm. degr. rate (h^{-1}): not measured Readily biodegradable	2	Misra *et al.* (1996)
	Degradation in liquid systems	Degradation in liquid-phase culture: Starting concentration: > 75 mg/L, complete degradation within 70 h	2	Misra & Pavlostathis (1997)
	Degradation in soil-slurry systems	Maximum soil-normalized biodegradation rate - in monoterpene mixture: 0.9 mg/L/h - as individual substance: 1.9 mg/L/h	2	Misra & Pavlostathis (1997)
α-Pinene	Unacclimated coniferous forest soil extracts, aerobic conditions at 23°C; Measured: biomass increase and headspace CO_2	Lag period (h): 200 Max. degr. rate (mg/L/h): 0.029 Norm. degr. rate (h^{-1}): not measured Ready biodegradable	2	Misra *et al.* (1996)
	Fate in wetland sediment	2004: 12240 ng/g 2005: 7890 ng/g (36 % decrease) 2006: 2640 ng/g (ca. 70 % decrease)	2	Palma-Fleming et al. (2013)

Substance	Study/endpoint type	Results	Reliability score	Reference
	Degradation in soil-slurry systems	Maximum soil-normalized biodegradation rate - in monoterpene mixture: 1.1 mg/L/h - as individual substance: 2.1 mg/L/h	2	Misra & Pavlostathis (1997)
	Liquid degradation by two bacterial species (*Pseudomonas fluorescens* and *Alcaligenes xylosoxidans*) tested singly and as consortium	Complete degradation (<0.l mg/L remaining) in 36 h by the consortium. After a 10-h lag period, a maximum rate of degradation of 3.6 mg/L/h was observed. The *A. xylosoxidans* isolate was shown to degrade α-Pinene to below 5 mg/L in the test system in 36 hours, achieving a maximum degradation rate of 3.6 mg/L/h. The *R. fluorescens* isolate showed little degradation of α-pinene until 36 h into the experiment and had a maximum degradation rate of 1.2 mg/L/h.	2	Kleinheinz *et al.* (1999)
γ-Terpinene	Unacclimated coniferous forest soil extracts, aerobic conditions at 23°C; Measured: biomass increase and headspace CO_2	Lag period (h): 168 Max. degr. rate (mg/L/h): 0.039 Norm. degr. rate (h^{-1}): not measured Ready biodegradable	2	Misra *et al.* (1996)
	Degradation in soil-slurry systems	Maximum soil-normalized biodegradation rate - in monoterpene mixture: 0.8 mg/L/h - as individual substance: 1.8 mg/L/h	2	Misra & Pavlostathis (1997)
α-Terpinolene	Unacclimated coniferous forest soil extracts, aerobic conditions at 23°C; Measured: biomass increase and headspace CO_2	Lag period (h): 174 Max. degr. rate (mg/L/h): 0.053 Norm. degr. rate (h^{-1}): not measured Ready biodegradable	2	Misra *et al.* (1996)
	Degradation in soil-slurry systems	Maximum soil-normalized biodegradation rate - in monoterpene mixture: 0.6 mg/L/h - as individual substance: 1.5 mg/L/h	2	Misra & Pavlostathis (1997)
α-Terpineol	Unacclimated coniferous forest soil extracts, aerobic conditions at 23°C; Measured: biomass increase and headspace CO_2	Lag period (h): 94 Max. degr. rate (mg/L/h): > 0,10 Norm. degr. rate (h^{-1}): not measured Ready biodegradable	2	Misra *et al.* (1996)

Substance	Study/endpoint type	Results	Reliability score	Reference
	Degradation in liquid systems	Degradation in liquid-phase culture: Starting concentration: 210 mg/L, complete degradation within 48 h	2	Misra & Pavlostathis (1997)
δ-Cadinene	Persistency and biodegradation, experimental data (OECD 301F test) vs. Catalogic estimation models	OECD test: > 60 % degradation in 28 days → not persistent Kinetic model: primary half-life 8 days, not persistent	1	Jenner *et al.* (2011)
Biotransformation				
Limonene	Biotransformation of limonene by *Pseudomonas putida*	Optimal degradation conditions at 30°C, pH 5 and 120 days. The bioconversion products were identified as perillyl alcohol and p-menth-1-ene-6,8-diol, and under optimum conditions the yields were 36% and 44%, respectively (a rate kinetic model indicated corresponding limiting yields of 44% and 56%). No further degradation of the products was observed using these bacteria.	2	Chatterjee & Bhattacharyya (2001)
	Anaerobic degradation by *Pseudomonas citronellolis*, using NO_3^- as e^--acceptor in enrichment culture	75 % of Limonene consumed, metabolite formed (traces): α-Terpinene	2	Harder & Probian (1995)
α-Terpineol	Metabolism by *Pseudomonas incognita*	P. incognita degrades α-Terpineol by at least three routes: - via oleuropeic acid - aromatization of α-Terpineol - formation of limonene	2	Madhava & Renganathan (1984)
	Anaerobic biotransformation (nitrate-reducing conditions, EtOH = e^--donor)	Decrease of 52 % after 7.5 d, remained constant until study termination (30 d)	2	Pavlostathis & Misra (1999)
p-Cymene	p-Cymene catabolic pathway in *Pseudomonas putida* F1	*P. putida* F1 utilizes p-Cymene by an 11-Step pathway through p-Cumate to isobutyrate, pyruvate, and acetyl CoA.	2	Eaton (1997)
α-Pinene	Anaerobic biotransformation (nitrate-reducing conditions, EtOH = e^--donor)	No significant degradation	2	Pavlostathis & Misra (1999)
	Bioconversion by *Pseudomonas* sp. strain PIN	Total bioconversion in 40 h: 33.5 % Formed products: Limonene, *p*-Cymene, *p*-Cymene-8-ol, α-Terpinolene, terpineol, camphor, terpinene-4-ol and borneol (all < 10 %)	2	Yoo *et al.* (2001)

Substance	Study/endpoint type	Results	Reliability score	Reference
	Bacterial metabolism by *Pseudomonas* sp. strain PIN	Substrate: α-Pinene (1 % v/v) Biomass: 103.13 g/L Maximum yield: 1056 mg/L Specific yield (cells): 10.24 Productivity: 22.00 mg/l h Formed metabolites: *p*-Cymene, Limonene, Trepineol, Terpinolene, Borneol	2	Yoo & Day (2002)
	Degradation by *Bacillus pallidus* BR425	Metabolites formed (all < 10 %): *β*-Pinene, Limonene, Pinocarveol, Pinocarvone, Myrtenol, Myrtenal, Carveol, Carvone	2	Savithiry et al. (1998)
	Anaerobic degradation by *Pseudomonas citronellolis*, using NO_3^- as e^--acceptor in enrichment culture	67 % of Limonene consumed, metabolites formed (traces): α-Terpinene, Cymene, Limonene, Eucalyptol	2	Harder & Probian (1995)
α-Pinene, p-Cymene 1,8-Cineole (=Eucalyptol), Terpinen-4-ol, α-Terpineol, Terpinolene, α- and γ-Terpinene	Mediterranean conditions; flowers of lavender oil.	Main substances were analysed monthly and after 12 months; p-Cymene and 1,8-Cineole were abundant in litter, and after 16 months 1,8-cineole. According to these investigations, the half-life rate of terpenes in litter is between 210 and 240 days under Mediterranean conditions. It should be noted that these are to be seen as worst case conditions for the Northern and Central Europe.	2	Hassiotis (2010)

Reliability statement: The literature studies from which the data listed in the above table are derived have not been performed according to official test guidelines. Therefore, it is not possible to evaluate their compliance by identifying any deviations or comparing them against guideline-specific quality criteria. Nonetheless, the publications in question have been assessed for relevance and scientific reliability and assigned a respective reliability score. Details of this process are described in the literature section (CA 9) of the active substance dossier, which clarifies that all studies used in the dossier were classified by the applicant as reliable or reliable with restrictions (supporting information).

11.1.2 BOD_5/COD

No data available.

11.1.3 Hydrolysis

An expert statement (Anonymous 2007a, Annex 3) concluded on following concerning hydrolysis and photochemical degradation of Tea Tree Oil:
Due to their high vapour pressure and rather low water solubility, especially of the terpene hydrocarbons, most the TTO constituents will volatilise from surface water within a very short time period after application. This is indicated by the high Henry constants and vapor pressures of the constituents.
Estimations of the photochemical oxidative degradation and experimental data show that all TTO constituents rapidly degrade in the gas phases under the influence of light.

Based on their low solubility in water, their high volatility and high reactivity under the influence of light, it may be assumed that the persistence time of the different TTO constituents in flora, soil and surface waters is rather low. Furthermore, based on the fact that the TTO constituents react rapidly with OH, NO_3 radicals and O_3 their residence time in the troposphere is also considered to be short.

These findings support the overall conclusion that Tea Tree Oil can be considered as rapidly degradable in light of hydrolysis.

11.1.4 Other convincing scientific evidence

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

TTO components are present in other environmental compartments due to the emission of terpenes and their redistribution (e.g. p-Cymene, gamma-Terpinene, alpha-Terpinene and Limonene) from rangeland and other crops. Emission from these natural sources results in background levels of terpene components in soil, water and sediment.

As these findings are not relevant for classification and labelling the respective information is not presented in the frame of the present assessment.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No data available and considered necessary.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

> *Study 1, Anonymous (2018f), [^{14}C]Terpinene-4-ol and gamma-Terpinene, p-Cymene, 1,8-Cineole, Globulol /Viridiflorol and Aromadendrene from Tea Tree Oil - Aerobic Degradation and Metabolism in two Water/Sediment Systems, OECD 308, GLP.*

Reliability statement: The study was conducted in line with OECD 121 and meets the respective validity criteria reported therein. Moreover, the method is considered suitable according to paragraph, applicability of the method‘ OECD 121, since it is considered particularly useful for volatile substances. The applied analytical method is fully validated in accordance with SANCO/3029/99 rev.4 (11/07/00). No relevant deviations from OECD 121 were identified. Hence, the study is considered reliable (reliability score: 1).

The route of [^{14}C]Terpinene-4-ol and rate of degradation of [^{14}C]Terpinene-4-ol, gamma-Terpinene, p-Cymene , 1,8-Cineole, (-)-Globulol / Viridiflorol and (+)-Aromadendrene from Tea Tree Oil were investigated under aerobic conditions at 20 °C in the dark using two different water/sediment systems (1WS Pfalz and 2WS Humsterbach). The study was performed with radio-labelled [^{14}C]Terpinene-4-ol and non-radio-labelled gamma-Terpinene, p-Cymene, 1,8-Cineole, (-)-Globulol / Viridiflorol and (+)-Aromadendrene from Tea Tree Oil over a period of 552 hours (23 days).
No metabolites > 10% were observed in this study.

> *Study 2, Anonymous (2018c), [^{14}C]Terpinene-4-ol and gamma-Terpinene, p-Cymene, 1,8-Cineole, Globulol /Viridiflorol and Aromadendrene from Tea Tree Oil - Aerobic Degradation in one Soil at 20 °C in the Dark, OECD 307, GLP.*

Reliability statement: The study was conducted in line with OECD 307 and meets the respective validity criteria reported therein. Moreover, the method is considered suitable according to point 5 in OECD 307, since

the analytical method was able to provide evidence that the tested substances could be kept in soil under the experimental conditions of the test and complete aerobic degradation could be demonstrated. The applied analytical method is fully validated in accordance with SANCO/3029/99 rev.4 (11/07/00). No relevant deviations from OECD 307 were identified. Hence, the study is considered reliable (reliability score: 1).

The route of [^{14}C]Terpinene-4-ol and rate of degradation of [^{14}C]Terpinene-4-ol, gamma-Terpinene, p-Cymene , 1,8-Cineole, (-)-Globulol / Viridiflorol and (+)-Aromadendrene from Tea Tree Oil were investigated under aerobic conditions at 20 °C in the dark in one soil of European origin (2.4). The study was performed with radio-labelled [^{14}C]Terpinene-4-ol and non-radio-labelled gamma-Terpinene, p-Cymene , 1,8-Cineole, ( ) Globulol / Viridiflorol and (+)-Aromadendrene from Tea Tree Oil over a period of 768 hours (32 days). [^{14}C]Terpinene-4-ol degraded to CO_2, non-extractable residues and minor metabolites. The DT_{50} values ranged from 0.9 to 151 hours.

Study 3, Anonymous (2018d), gamma-Terpinene, p-Cymene, 1,8-Cineole, Globulol / Viridiflorol and Aromadendrene - Aerobic Degradation in Three Soils at 20 °C in the Dark, OCED 307, GLP.

Reliability statement: The study was conducted in line with OECD 307 and meets the respective validity criteria reported therein. Moreover, the method is considered suitable according to point 5 in OECD 307, since the analytical method was able to provide evidence that the tested substances could be kept in soil under the experimental conditions of the test and complete aerobic degradation could be demonstrated. The applied analytical method is fully validated in accordance with SANCO/3029/99 rev.4 (11/07/00). No relevant deviations from OECD 307 were identified. Hence, the study is considered reliable (reliability score: 1).

The rate of degradation of gamma-Terpinene, p-Cymene, 1,8-Cineole, Globulol / Viridiflorol and Aromadendrene was investigated under aerobic conditions at 20 ± 2 °C in the dark using three soils of European origin (LUFA 2.1, LUFA 2.2 and LUFA 6S). The study was performed over a period of 32 days.

In the aerobic soil degradation study, 10 minor transformation products evolved. None of the metabolites reached 5 % AR. The DT_{50} values ranged from 1.6 to 262 hours.

The results from the water-sediment study in two different aquatic systems and from the soil degradation studies indicate that the components of Tea Tree Oil degrade rapidly in the tested environmental compartments.

11.1.4.4 Photochemical degradation

None of the Components, with the exception of p-Cymene (max. 8% of total) and α-terpinene (max. 13% of total), contains conjugated double bonds. Accordingly, none of these substances have significant absorption at 290 nm or above.

Therefore, direct aqueous photolysis is at most relevant only for p-Cymene. However, the spectra for p-Cymene in Roehri, C. (2017) clearly show that p-Cymene does not absorb at wavelength (λ) ≥ 290 nm. Since p-Cymene has 3 conjugated double bonds (aromatic ring) and and α-terpinene only 2 conjugated double bonds, it is therefore clear that α-terpinene also will not absorb at wavelength (λ) ≥ 290 nm since absorption at higher wavelengths requires more conjugated double bonds.

Therefore, no studies on direct photochemical degradation are deemed necessary.

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant for classification of Tea Tree Oil.

11.2.1 Summary of data/information on environmental transformation

Not relevant for classification of Tea Tree Oil.

11.3 Environmental fate and other relevant information

Table 67: Adsorption and desorption of the active substance

<table>
<tr><th>Study</th><th>Method / Results</th><th>Remarks</th><th>Reliability score</th><th>Reference</th></tr>
<tr><td colspan="5">Adsorption and desorption of the active substance</td></tr>
<tr><td>Estimation of Adsorption-Coefficient on Soil of Terpinene-4-ol, gamma-Terpinene, p-Cymene, 1,8-Cineole, Globulol / Viridiflorol and Aromadendrene using High Performance Liquid Chromatography (HPLC)
OECD 121
GLP</td><td>The aim of this study was the determination of the adsorption coefficient of Terpinene-4-ol, gamma-Terpinene, p Cymene, 1,8-Cineole, Globulol / Viridiflorol and Aromadendrene on soil and on sewage sludge (KOC) by high-performance liquid chromatography (HPLC) method according to EC method C.19 (440/2008) and OECD guideline 121.
Results:
<table>
<tr><th>Test Item</th><th>Mean retention time of two runs [min]</th><th>Absolute deviation [min]</th><th>Mean log K_{OC} of two runs</th><th>Absolute deviation of log K_{OC}</th></tr>
<tr><td>Terpinene-4-ol</td><td>3.40</td><td>0.01</td><td>1.95</td><td>0.00</td></tr>
<tr><td>gamma-Terpinene</td><td>6.06</td><td>0.03</td><td>3.36</td><td>0.01</td></tr>
<tr><td>p-Cymene</td><td>5.46</td><td>0.02</td><td>3.13</td><td>0.01</td></tr>
<tr><td>1,8-Cineole</td><td>3.19</td><td>0.01</td><td>1.77</td><td>0.00</td></tr>
<tr><td>(-)-Globulol</td><td>5.74</td><td>0.01</td><td>3.24</td><td>0.00</td></tr>
<tr><td>(+)-Aromadendrene</td><td>13.88</td><td>0.08</td><td>> 5.0 (5.05)*</td><td>0.00</td></tr>
</table>
* above the determination limit of the method (5.0 according to OECD 121) but within the calibration range (log K_{OC} of the highest reference standard 4,4'-DDT is 5.63)</td><td></td><td>1</td><td>Anonymous (2018e)</td></tr>
<tr><td>None, statement

Direct photochemical degradation</td><td>None of the Components, with the exception of p-Cymene (max. 8% of total), contains conjugated double bonds. Accordingly, none of these substances have significant absorption at 290 nm or above.
Therefore, direct aqueous photolysis is at most relevant only for p-Cymene. However, the spectra for p-Cymene in Roehri, C. (2017) clearly show that p-Cymene does not absorb at wavelength (λ) ≥ 290 nm.</td><td></td><td></td><td></td></tr>
</table>

Mobility in soil:

Study 1, Anonymous (2018e), Estimation of Adsorption-Coefficient on Soil of Terpinene-4-ol, gamma-Terpinene, p-Cymene, 1,8-Cineole, Globulol / Viridiflorol and Aromadendrene using High Performance Liquid Chromatography (HPLC), OECD 121, GLP.

Reliability statement: The study was conducted in line with OECD 121 and meets the respective validity criteria reported therein. Moreover, the method is considered suitable according to paragraph ‚applicability of the method' OECD 121, since it is considered particularly useful for volatile substances. The applied analytical method is fully validated in accordance with SANCO/3029/99 rev.4 (11/07/00). No relevant deviations from OECD 121 were identified. Hence, the study is considered reliable (reliability score: 1).

As for the components of Tea Tree Oil, the batch equilibrium method cannot be applied due to fast degradation, the HPLC (high-performance liquid chromatography) method was considered as a possible alternative.

For the components of Tea Tree Oil, mean log K_{OC} values of 1.77 – 5.05 were derived from two measurements, corresponding to K_{OC} values of 58.9 for 1,8-Cineole to 112201 for (+)-Aromadendrene.

Direct photochemical degradation

None of the components of TTO would be expected to be transported in the gaseous phase over large distances or to accumulate in the air.

11.4 Bioaccumulation

Table 68: Summary of relevant information on bioaccumulation

<table>
<tr><th>Method</th><th>Results</th><th>Reference</th></tr>
<tr><td>Measured</td><td>Terpinen-4-ol
Log P_{OW} = 2.643 at 23.5°C and pH of 5.85.</td><td>Parsons, A. 2007</td></tr>
<tr><td>Experimental and estimated values</td><td>
<table>
<tr><th>Components</th><th>BCF</th><th>Log Pow</th></tr>
<tr><td>Terpinen-4-ol</td><td>66**</td><td>2.80[3] ;</td></tr>
<tr><td>α-Pinene</td><td>395**</td><td>4.83[1]; 4.48[3]</td></tr>
<tr><td>Limonene</td><td>360**</td><td>4.57[1]; 4.38[3]</td></tr>
<tr><td>γ-Terpinene</td><td>433**</td><td>4.50[1]; 4.36[3]</td></tr>
<tr><td>Terpinolene</td><td>296**</td><td>4.47[1]; 4.24[3]</td></tr>
<tr><td>α-Terpineol</td><td>68**</td><td>2.98[1]; 3.28[3]</td></tr>
<tr><td>p-Cymene</td><td>236**</td><td>6.34[2]; 4.10*</td></tr>
<tr><td>α-Terpinene</td><td>433**</td><td>4.25[3]</td></tr>
<tr><td>1,8-Cineole</td><td>30**</td><td>2.74[3]</td></tr>
<tr><td>Aromadendrene</td><td>5129**</td><td>6.13**</td></tr>
<tr><td>δ-Cadinene</td><td>6838**</td><td>6.32**</td></tr>
<tr><td>Sabinene</td><td>577**</td><td>4.69**</td></tr>
<tr><td>Globulol</td><td>529**</td><td>4.63**</td></tr>
<tr><td>Viridiflorol</td><td>529**</td><td>4.63**</td></tr>
<tr><td>Ledene</td><td>5543**</td><td>6.18**</td></tr>
</table>
[1] Experimental value from Li & Perdue (1998)
[2] Experimental value from Banerjee *et. al* 1980
[3] Experimental value from Griffin *et al.* 1999
*From Episuite v4.11, experimental database match
** From Episuite v4.11, estimated
</td><td>Li, J. Perdure, E.M. et al., 1998

Banerjee, S.; Yalkowsky, S.H. & Valvani, S.C. 1980

Griffin, S.; Wyllie, S.G. & Markham, J., 1999</td></tr>
</table>

11.4.1 Estimated bioaccumulation

Estimated BCF is < 500 for all monoterpene components, which account on average for > 95% of Tea Tree Oil. For the sesquiterpenes, BCF > 500 has been estimated, however, for the majority of these below 600, i.e. close to the trigger of 500. The sesquiterpene content of Tea tree oil is traces to max. 3.5% (individually), and cumulatively usually < 5%. Cumulative content of components with BCF > 600 (Cadinene, Aromadendrene and Ledene, BCF range 5000-7000) usually is below 2%.

Overall, Tea Tree Oil is considered to be not potentially bioaccumulative.

11.4.2 Measured partition coefficient and bioaccumulation test data

The experimental logP_{OW} of Terpinen-4-ol, the main and representative component of Tea Tree Oil amounts to 2.643 at 23.5° and pH 5.85 and thus does not exceed the trigger value of 4 (logP_{OW} < 4).

There are no bioaccumulation test data available.

11.5 Acute aquatic hazard

A summary of all the relevant and reliable information on the acute aquatic toxicity of Tea Tree Oil is presented in Table 69. Studies were conducted according to internationally agreed standard test guidelines and corresponding validity criteria were met. In the following sections, executive summaries of the available studies on Tea Tree Oil and its components are provided that give more detailed information on acute aquatic toxicity.

Table 69: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results [mg as/L]	Remarks	Reliability score	Reference
Acute toxicity to fish OECD 203, GLP Semi static, 96 h	*Oncorhynchus mykiss*	Tea Tree Oil	LC_{50} = 7.45	measured	1	Anonymous (2015f)
Acute toxicity to fish OECD 203 96 h semi-static	*Brachydanio rerio*	Tea Tree Oil	LC_{50} > 100 NOEC = 100	nominal	3	Anonymous (1999a)
Literature study 96 h static Fish toxicity test	*Oncorhynchus mykiss*	α-Terpineol	LC_{50} = 6.6	measured	3	Stroh, J. et al (1998)
	Oncorhynchus kisutch	α-Terpineol	LC_{50} = 6.3	measured	3	Stroh, J. et al (1998)
Literature study US EPA 660/3-75-009 (1975) guideline 96 h static	*Cyprinodon variegatus*	p-Cymene	LC_{50} = 48 NOEC = 10	measured	3	Heitmuller, P.T. et al (1981)
Acute toxicity to daphnia OECD 202, GLP Semi-static, 48 h	*Daphnia magna*	Tea Tree Oil	EC_{50} = 0.591 NOEC = 0.106	measured	1	Anonymous (2011a)
Literature study Acute toxicity to daphnia US EPA 660/3-75-	*Daphnia magna*	p-Cymene	EC_{50} = 6.5 NOEC < 4.6	nominal	3	LeBlanc, G.A. (1980)
	Daphnia magna	α-Pinene	LC_{50} = 41 NOEC = 8.8	nominal	3	LeBlanc, G.A. (1980)

009 guideline Static, 48 h						
Algae growth inhibition test OECD 201, GLP	*Pseudokirchneriella subcapitata*	Static, 72 h Tea Tree Oil	E_yC_{50} = 1.76 E_rC_{50} = 2.17	measured	1	Anonymous (2017c)
Aquatic plant toxicity test OECD 221, GLP	*Lemna gibba*	Semi-static, 7 d Tea Tree Oil	E_rC_{50} = 10.3 E_yC_{50} = 10.0	measured	1	Anonymous (2017d)

11.5.1 Acute (short-term) toxicity to fish

Two acute toxicity studies on Tea Tree Oil showed short-term (96 hour) acute toxicity to fish in *Oncorhynchus mykiss* and *Brachydanio rerio*. 96 hour LC_{50} values were 7.45 mg as/L in *Oncorhynchus mykiss* and > 100 mg as/L in *Brachydanio rerio*.

Two studies on the component of Tea Tree Oil α-Terpineol on *Oncorhynchus mykiss* and *Oncorhynchus kisutch* lead to 96 hour LC_{50} values of 6.6 and 6.3 mg as/L, respectively.

One study on *Cyprinodon variegatus* with the component of Tea Tree Oil p-Cymene lead to a 96h LC_{50} value of 48 mg as/L.

Study 1: Anonymous (2015f), Extract from Tea Tree (Tee Tree Oil). Fish (Rainbow trout), acute toxicity test, semi-static, 96 h, OECD 203, GLP

Reliability statement: The study was performed in line with recommendation of OECD 203 with no major deviations. The validity criteria regarding oxygen concentration, environmental conditions and mortality in control groups were fulfilled and the study is considered acceptable (reliability score: 1).

The acute toxicity of Tea Tree Oil to Rainbow trout was determined in fresh water at 14°C using a semi-static 96 h test system at nominal concentrations of 1.71, 3.76, 8.26, 18.2 and 40.0 mg TTO/L.

Records of mortality and sublethal effects of exposure were made at 24, 48, 72 and 96 hours after the start of the exposure.

No mortality occurred at the three lowest test concentrations during the whole period of exposure of 96 h. A 100 % mortality was recorded at the highest tested concentration of 40 mg TTO/L.

The 96-hour LC_{50} for the rainbow trout was 7.45 mg TTO/L (95 % confidence limits: 3.85 to 7.64 mg/L), based on geometric mean measured concentrations.

Table 70: Observed mortality of fish exposed to Tea Tree Oil for 96 hours

Nominal concentration of Tea Tree Oil [mg/L]	Cumulative Mortality [%]				
	2 h	24 h	48 h	72 h	96 h
40	0	100	100	100	100
18.2	0	14	57	71	71
8.26	0	0	0	0	0
3.76	0	0	0	0	0
1.71	0	0	0	0	0
Control	0	0	0	0	0

Study 2: Anonymous (1999a) , Main Camp Tea Tree Oil pharmaceutical grade: fish, acute toxicity test, OECD 203, pre-GLP

Reliability statement: Very limited information is presented in the study report, which seems to be rather summary than actual test report. No information regarding fish size is available. No information regarding purity of the test item is given (e.g. concentration of the "lead components"). Sub-lethal effects are not reported, but from the available information it cannot be deduced if they have not occurred or were not investigated.

The most significant deficiency of the study is lack of the verification of the test item concentrations in any of the freshly prepared or old test solutions. For this reason it cannot be confirmed if the nominal concentrations were maintained at ±20% of nominal, but it seems to be highly unlikely given the way of preparation of test solutions - due to stirring of the stock solution for 24 hours it may be expected that at least part of the components of the extract volatilised. Furthermore, the undissolved phase was removed and only the dissolved water phase was used in the study, so fish were exposed only to part of components of the tea tree extract.

Overall, taking into account deficiencies mentioned above, the study is considered unacceptable (reliability score: 3).

The acute toxicity of Tea Tree Oil to zebra fish (*Brachydanio rerio*) has been conducted in a 96-hour semi-static design according to OECD Guideline 203. Ten fish were exposed to the concentrations of 5, 10, 25, 50 and 100 mg Tea Tree Oil/L.

No mortality occurred throughout the exposure period of 96 h.

The 96-hour LC_{50} and NOEC were determined to be > 100 mg Tea Tree Oil/L, based on the nominal concentrations.

Study 3: Stroh, J. et al., 1998; Literature study (Evaluation of the acute toxicity to juvenile pacific coho salmon and rainbow trout of some plant essential oils, a formulated product, and the carrier; Bull. Environ. Contam. Toxicol. 60:923-930)

Reliability statement: This public literature study was performed in line with protocol outlined in Wan et al. (1990, 1991) and Environment Canada (1990a, 1990b). Description of the test methods shows that the test design partially followed recommendations of OECD 203.

However, important information is missing in the paper, e.g.:

- size of the fish at test initiation,
- exact test concentrations,
- more detailed data regarding test conditions (especially oxygen concentration, which is a test validity criterion),
- preparation of test solutions,
- numerical data on mortality in each test group including controls.

In addition to that, results of chemical analyses are reported only for eugenol with no information regarding

measured concentrations of α-terpineol. Based on obtained results, the study authors concluded that the total loss of the test item from test solutions could be 90% of nominal. However, results of the test are based on nominal concentrations.

Overall, as the measured concentrations of α-terpineol were not presented and due to not sufficient reporting it cannot be confirmed if validity criteria of OECD 203 were fulfilled, the study is considered as unreliable and its results cannot be used in the regulatory risk assessment (reliability score: 3).

The acute toxicity of α-terpineol to juvenile (2-3 months old) rainbow trout (*Oncorhynchus mykiss*), and pacific coho salmon (*Oncorhynchus kisutch*) was determined in a 96-hour static test. Glass aquaria of 20 L volume served as test vessels, resulting in an average loading rate of 0.4 g fish/L. Fish were exposed to a series of 5

test concentrations below 100 mg α-terpineol/L. Each test concentration was tested in triplicate with ten fish per test vessel. Test media were slightly aerated throughout the test. Fresh ground water with a mean hardness of 95 mg/L $CaCO_3$ was used for dilution water. Testing was carried out at 15 ± 1 °C. Conductivity, pH, dissolved oxygen concentration, and temperature were measured frequently throughout the test.

Test substance concentrations during test: to evaluate chemical loss in the test materials, water samples were taken from control and treatment vessels containing 18 ppm (nominal) blended product (thyme oil: alpha-Terpineol: Eugenol (1:1:1)). Not measured for test substance α-terpineol vessels separately, just for blended product.

No data of these measurements given, but stated that there was a chemical loss in all tested vessels with and without fish and that it was more than 90% of the nominal concentration. This loss of test chemicals was explained by volatilization during the initial aeration process and glass adsorption. However, it was unclear if this statement referred only to the substance eugenol or also to alpha-terpineol. No fish tissue analyses were conducted after the test.

LC_{50} values of 6.6 and 6.3 mg alpha-Terpineol/L were determined for the rainbow trout (*Oncorhynchus mykiss*) and the pacific coho salmon (*Oncorhynchus kisutch*), respectively. Please refer also to the table below for more details on the results.

Table 71: Results of the calculated LC_{50} values

Test species	**LC_{50} [mg α-terpineol/L]**			
	24 h	**48 h**	**72 h**	**96 h**
O. mykiss	6.7	6.7	6.6	6.6
O. kisutch	6.8	6.5	6.5	6.3

Study 4: Heitmuller, P.T. et al (1981); Literature study (Acute toxicity of 54 industrial chemicals to sheepshead minnow (Cyprinodon variegatus). Bull. Environ. Contam. Toxicol. 27, 596-604)

Reliability statement: This public literature study was performed in line with US EPA guideline. Description of the test methods shows that the test design partially followed recommendations of OCSPP 850.1075 of 2016.
However, important information is missing in the paper, e.g.:

- exact test concentrations,
- whether fish were fed or not (it may be deduced that fish were not fed, but this is not explicitly indicated),
- more detailed data regarding test conditions (especially oxygen concentration, which is a test validity criterion),
- numerical data on mortality in each test group including controls.

Furthermore, in the publication it is stated that:
Many of the chemicals were insoluble in seawater and either floated upon the water surface or formed globules on the bottoms of the test containers.
It is, however, not indicated, which chemicals were insoluble.
In addition to that, chemical analyses were not performed and for this reason it is not known if the test concentrations were maintained at required ±20% of nominal.
Overall, as the measured concentrations of p-cymene were not presented and due to not sufficient reporting it cannot be confirmed if validity criteria of OCSPP 850.1075 were fulfilled, the study is considered as unreliable and its results cannot be used in the regulatory risk assessment (reliability score: 3).

The acute toxicity of p-Cymene to unfed sheepshead minnow (*Cyprinodon variegatus*) 14 to 28 days old, post hatch, length of 8 to 15 mm) was determined in a static, 96- hour test according to the US EPA 660/3-75-009 (1975) guidelines.

Chemicals tested were analytical grade with a minimum purity of 80%. There was no aeration. Filtered natural seawater was used as dilution water. In the separate tests, a stock solution was prepared using a solvent (triethylene or acetone) or the substance was added directly to dilution water.

Treatments consisted of a series of concentrations (actual values not indicated), a dilution water control and/or a solvent control when necessary. 10 fish were used per treatment level. Tests were conducted in either 4-L glass jars containing 3 L of test medium or 19-L glass jars containing 15 L of test medium.

Test substance concentrations were not determined in the test media. Water quality parameters dissolved oxygen and pH were determined during testing; actual values are not indicated in the published report.

It has been stated that results were not considered acceptable if control mortality exceeded 10%.

The LC_{50} value of 48 mg p-Cymene/L was determined for sheepshead minnow (*Cyprinodon variegatus*). Please refer also to the table below for more details on the results.

Table 72: Results of the calculated LC_{50} values

LC_{50} (95 % confidence interval) **[mg p-Cymene/L]**				**NOEC [mg p-Cymene/L]**
24 h	**48 h**	**72 h**	**96 h**	
56 (32 – 100)	50 (38 – 68)	48 (36 – 64)	48 (36 – 64)	10

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

One acute toxicity study with Tea Tree Oil showed short-term (48 hour) acute toxicity to *daphnia* with an EC_{50} value of 0.591 mg as/L.

Two acute toxicity studies on the components of Tea Tree Oil p-Cymene and α-Pinene revealed short-term (48 hour) acute toxicity to *daphnia* with EC_{50} values of 6.5 and 41 mg as/L, respectively.

Study 1: Anonymous (2011a), Tea Tree Oil: Acute immobilisation test to Daphnia magna, semi-static, 48 hours, OECD 202, GLP

Reliability statement: The study was performed in line with recommendations of OECD 202 with no major deviation. All validity criteria were met and the study is considered acceptable (reliability score: 1).

In order to investigate the acute toxicity of Tea Tree Oil in aquatic invertebrates, the daphnid *Daphnia magna* was exposed over a period of 48 h under semi-static conditions to 6 concentrations of the test item ranging from 0.250 to 8.00 mg/L set up in a geometric series with a dilution factor of 2. Twenty (20) test organisms were exposed to each concentration and control.

The 48-hour EC_{50} value for *Daphnia magna* for Tea Tree Oil was determined to be 0.591 mg/L (95% confidence limits: 0.499 – 0.700 mg/L) based on measured concentrations. The no observed effect concentration (NOEC) was 0.106 mg/L based on measured concentrations.

Table 73: Summary of observed immobility of *Daphnia magna* exposed to Tea Tree Oil for 48 hours in semi-static, acute test

Geometric mean measured Concentrations of test item [mg/L]	**Number of *Daphnia magna***	**Mean Immobilization [%] Time**	
		24 h	**48 h**
2.65	20	75	100
1.60	20	45	95
0.750	20	30	60
0.374	20	0	25
0.169	20	5	15
0.106	20	0	0
Control	20	0	0

Study 2: LeBlanc, G.A. 1980, Literature study (Acute toxicity of priority pollutants to water flea (Daphnia magna); Bull. Environ. Contam. Toxicol. 27, 684-691) LeBlanc, G.A., 1980

Reliability statement: This public literature study was performed in line with US EPA guideline. Description of the test methods shows that the test design partially followed recommendations of OCSPP 850.1010 of 2016.
However, important information is missing in the paper, e.g.:

- exact test concentrations,
- solvent used to dissolve p-cymene and α-pinene in water (if any),
- numerical data on immobilisation in each test group including controls.

Furthermore, 15 daphnids per concentration were used, while according to the guideline minimum 20 daphnids should be used per test group.
In addition to that, chemical analyses were not performed and for this reason it is not known if the test concentrations were maintained at required ±20% of nominal.
Overall, as the measured concentrations of p-cymene and α-pinene were not presented and due to not sufficient reporting it cannot be confirmed if validity criteria of OCSPP 850.1010 were fulfilled, the study is considered as unreliable and its results cannot be used in the regulatory risk assessment (reliability score: 3).

The acute toxicity of p-Cymene and α-Pinene to unfed *Daphnia magna* (< 24 hours old) was determined separately in static, 48-hour tests according to the US EPA 660/3-75-009 guidelines. Reconstituted water was used as dilution water. Depending upon solubility, either a stock solution was prepared with or without using a solvent (triethylene, glycol, ethanol, acetone, or dimethylformamide) or the substance was added directly to dilution water. Using the corresponding method, the test substance was added to 500 mL of dilution water in a 2 L jar to prepare each test solution. Depending upon solubility and volatility, the test solution was either divided in three 150 mL aliquots to provide three replicates or retained as it was to omit substance losses due to handling.

Treatments consisted of a series of 5 to 8 concentrations (actual values not indicated), a dilution water control and a solvent control when necessary. 15 daphnids were used per treatment level. For non-soluble substances that may be lost through volatilisation, one closed test vessel with 15 daphnids per treatment level was used. Tests were conducted at 22 ± 1°C. Test substance concentrations were not determined in the test media.

Control mortality was below 10% during this study.

The 48-hour EC_{50} for *Daphnia magna* was determined to be 6.5 mg/L for p-Cymene and 41 mg/L for alpha-Pinene, based on nominal concentrations.

Table 74: Results of the calculated LC_{50} values

Test substance	LC_{50} (95 % confidence interval) [mg a.i./L]		NOEC [mg a.i./L]
	24 h	48 h	
p-Cymene	9.4 (7.9 -11)	6.5 (4.3 - 10)	< 4.6
α-Pinene	68 (24 – 190)	41 (27 – 62)	8.8

In the table below, data from the open scientific literature is presented as an overview. These data are regarded solely as supplemental information.

Table 75: Open scientific literature data on effects of TTO or its components on aquatic invertebrates

Substance	Species	Results	Analytical verification of test item	Reliability score*	Reference
α-Pinene	*Aedes aegypti* larvae	48 h Mortality [%]: 40.0 ± 8.1 at 100 mg/L	Yes	2	Park et al. (2011)

Substance	Species	Results	Analytical verification of test item	Reliability score*	Reference
		24h LC_{50}: > 50 mg/L	No	3	Cheng et al. (2009)
		24h LC_{50}: 15.87 mg/L	No	3	Lucia et al. (2013)
		24h LC_{50}: > 100 mg/L	No	3	Cheng et al. (2013)
		24h LC_{50}: 15.4 mg/L	No	3	Lucia et al. (2007)
	Aedes albopictus larvae	24h LC_{50} (+): 68.68 mg/L 24h LC_{50} (-): 72.30 mg/L	No	3	Giatropoulos et al (2012)
		24h LC_{50}: > 50 mg/L	Yes	3	Cheng et al. (2009)
		24h LC_{50}: > 100 mg/L	Yes	3	Cheng et al. (2013)
		24h LC_{50}: 34.09 mg/L	No	3	Govindarajan et al. (2016)
	Anopheles suspictus larvae	24h LC_{50}: 32.09 mg/L	No	3	Govindarajan et al. (2016)
	Culex quinquefasciatus larvae	24h LC_{50}: 95 mg/L	No	3	Pavela (2015)
α-Terpinene	*Daphnia magna*	48h LC_{50}, 8.45 mg/L	Yes	2	Park et al. (2011)
	Aedes aegypti larvae	48 h Mortality [%]: 100 at 25.0 mg/L 5.0 ± 2.8 at 12.5 mg/L	Yes	2	Park et al. (2011)
		24h LC_{50}: 28.1 mg/L	No	3	Cheng et al. (2009)
	Aedes albopictus larvae	24h LC_{50}: 22.4 mg/L	No	3	Cheng et al. (2009)
	Culex quinquefasciatus larvae	24h LC_{50}: > 250 mg/L	No	3	Pavela (2015)
	Culex tritaeniorhynchus larvae	24h LC_{50}: 36.75 mg/L	No	3	Govindarajan et al. (2016)
p-Cymene	*Daphnia magna*	48h LC_{50}, 3.54 mg/L	Yes	2	Park et al. (2011)
	Aedes aegypti larvae	48 h Mortality [%]: 100 at 50 mg/L 5.0 ± 5.0 at 25 mg/L	Yes	2	Park et al. (2011)
		24h LC_{50}: 43.3 mg/L	No	3	Cheng et al. (2009)

Substance	Species	Results	Analytical verification of test item	Reliability score*	Reference
		24h LC_{50}: 12.49 mg/L	No	3	Lucia et al. (2013)
		24h LC_{50}: 69.4 mg/L	No	3	Cheng et al. (2013)
	Aedes albopictus larvae	24h LC_{50}: 34.9 mg/L	No	3	Cheng et al. (2009)
		24h LC_{50}: 68.3 mg/L	No	3	Cheng et al. (2013)
	Culex quinquefasciatus larvae	24h LC_{50}: 21 mg/L	No	3	Pavela (2015)
		24 h LC_{50}: 20.6 µl/L**	No	3	Pavela et al. (2017)
(-)-Limonene	*Daphnia magna*	48h LC_{50}, 7.22 mg/L	Yes	2	Park et al. (2011)
	Aedes aegypti larvae	48 h Mortality [%]: 100 at 100 mg/L 97.5 ± 2.5 at 50 mg/L 32.5 ± 10.3 at 25 mg/L	Yes	2	Park et al. (2011)
	Aedes albopictus larvae	24h LC_{50}: 34.89 mg/L	No	3	Giatropoulos et al (2012)
(+)-Limonene	*Daphnia magna*	48h LC_{50}, 7.85 mg/L	Yes	2	Park et al. (2011)
	Aedes aegypti larvae	48 h Mortality [%]: 100 at 100 mg/L 50.0 ± 9.1 at 50 mg/L 10.0 ± 4.0 at 25 mg/L	Yes	2	Park et al. (2011)
		24h LC_{50}: 19.4 mg/L	No	3	Cheng et al. (2009)
		24h LC_{50}: 39.7 mg/L	No	3	Chung et al. (2010)***
		24h LC_{50}: 71.9 mg/L	No	3	Cheng et al. (2013)
		24h LC_{50}: 29.1 mg/L	No	3	Tabanca et al. (2015)
	Aedes albopictus larvae	24h LC_{50} (+): 35.99 mg/L	No	3	Giatropoulos et al (2012)
		24h LC_{50}: 15.0 mg/L	No	3	Cheng et al. (2009)
		24h LC_{50}: 19.84 mg/L	No	3	Liu et al. (2013)
		24h LC_{50}: 41.75 mg/L	No	3	Liu et al. (2015)

Substance	Species	Results	Analytical verification of test item	Reliability score*	Reference
		24h LC_{50}: 41.2 mg/L	No	3	Cheng et al. (2013)
	Culex quinquefasciatus larvae	24h LC_{50}: 40 mg/L	No	3	Pavela (2015)
γ-Terpinene	*Daphnia magna*	48h LC_{50}, 3.45 mg/L	Yes	2	Park et al. (2011)
	Aedes aegypti larvae	48 h Mortality [%]: 100 at 50 mg/L 7.5 ± 2.5 at 25 mg/L	Yes	2	Park et al. (2011)
		24h LC_{50}: 26.8 mg/L	No	3	Cheng et al. (2009)
		24h LC_{50}: 0.4 mg/L	No	3	Lucia et al. (2013)
	Aedes albopictus larvae	24h LC_{50}: 20.21 mg/L	No	3	Giatropoulos et al (2012)
		24h LC_{50}: 22.8 mg/L	No	3	Cheng et al. (2009)
	Culex quinquefasciatus larvae	24h LC_{50}: 26 mg/L	No	3	Pavela (2015)
Terpinolene	*Aedes aegypti* larvae	48 h Mortality [%]: 100 at 100 mg/L 97.5 ± 2.5 at 50 mg/L 32.5 ± 10.3 at 25 mg/L	Yes	2	Park et al. (2011)
		24h LC_{50}: 32.1 mg/L	No	3	Cheng et al. (2009)
	Aedes albopictus larvae	24h LC_{50}: 21.3 mg/L	No	3	Cheng et al. (2009)
	Culex quinquefasciatus larvae	24h LC_{50}: 21 mg/L	No	3	Pavela (2015)
		24 h LC_{50}: 25.7 µl/L**	Yes	2	Pavela et al. (2017)
Terpinene-4-ol	*Aedes aegypti* larvae	48 h Mortality [%]: 0 at 100 mg/L, lower concentrations not tested	Yes	2	Park et al. (2011)
		24h LC_{50}: 38.77 mg/L	No	3	Lucia et al. (2013)
α-Terpineol	*Aedes aegypti* larvae	48 h Mortality [%]: 2.5 ± 2.5 at 100 mg/L	Yes	2	Park et al. (2011)
		24h LC_{50}: 76.68 mg/L	No	3	Lucia et al. (2013)

Substance	Species	Results	Analytical verification of test item	Reliability score*	Reference
		24h LC_{50}: 331.7 mg/L	No	3	Pandey et al. (2013)
		24h LC_{50}: > 100 mg/L	No	3	Cheng et al. (2013)
	Aedes albopictus larvae	24h LC_{50}: > 100 mg/L	No	3	Cheng et al. (2013)
	Culex quinquefasciatus larvae	24h LC_{50}: > 250 mg/L	No	3	Pavela (2015)
1,8-Cineole	*Aedes aegypti* larvae	24h LC_{50}: 53.63 mg/L	No	3	Lucia et al. (2013)
		24h LC_{50}: > 200 mg/L	No	3	Chung et al. (2010)***
		24h LC_{50}: 57.2 mg/L	Yes	2	Lucia et al. (2007)
	Culex pipiens	48h LC_{50}: > 200 mg/L	No	3	Koliopoulos et al (2010)
		24h LC_{50}: 105.6 mg/L	No	3	Kimbaris et al (2012)
	Culex quinquefasciatus larvae	24h LC_{50}: > 250 mg/L	No	3	Pavela (2015)
Sabinene	*Culex quinquefasciatus* larvae	24h LC_{50}: 25.01 mg/L	No	3	Govindarajan (2010)
		24 h LC_{50}: 57.7 µl/L**	No	3	Pavela et al. (2017)
	Aedes aegypti larvae	24h LC_{50}: 21.20 mg/L	No	3	Govindarajan (2010)
		24h LC_{50}: 74.1 mg/L	No	3	Cheng et al. (2013)
	Aedes albopictus larvae	24h LC_{50}: 39.5 mg/L	No	3	Cheng et al. (2013)
	Anopheles stephensi larvae	24h LC_{50}: 19.67 mg/L	No	3	Govindarajan (2010)

* as usual for scientific literature, the testing was not conducted to fulfil regulatory requirements. Where reliability score 2 was assigned, analytical determination was conducted in any way, mostly to determine thepresence of the test substance in the mixture, but not for verification of the test item concentration. This data is considered as supportive information. Where reliability score 3 was assigned, no analytical validation and measurement was conducted. These publications can only be considered as additional information.

** concentration in mg/L cannot be concluded from information given in the publication.

*** publication has been retracted by the author subsequently, since deemed not reliable

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

An acute algae growth inhibition test with Tea Tree Oil with *Pseudokirchneriella subcapitata* is available. It lead to an E_yC_{50} of 1.76 mg as/L, an E_rC_{50} of 2.17 mg as/L with a NOEC of 0.912 mg as/L.

The semi-static 7d aquatic plant toxicity test with *Lemna gibba* resulted to a E_rC_{50} of 10.3 mg as/L, an E_yC_{50} of 10.0 mg as/L and a NOEC of 1.91 mg as/L.

Study 1: Anonymous (2017d) Tea Tree Oil (TTO): Aquatic plant toxicity test, Lemna gibba, semi-static, 7 days, OECD 221, GLP

Reliability statement: The study was performed in line with OECD 211 with no major deviations. All validity criteria were met and the test is considered acceptable (reliability score: 1)

The effects of Tea Tree Oil on the growth of the monocotyledonous aquatic plant species *Lemna gibba* applied at 5 dose rates 6.25, 12.5, 25.0, 50.0 and 100 mg Tea Tree Oil/L (nominal) was observed over a period for 7 days in a semi-static test system. Three replicates at each test concentration and six replicates for the control were included in the test. 2 additional replicates were treated at the highest concentration level and the control, respectively, and incubated and were used for the test on the recovery of the test system.

Frond numbers were assessed on days 0, 2, 5 and 7.

The 7-day E_yC_{50} value for Tea Tree Oil was determined to be 10.0 mg Tea Tree Oil/L and 10.6 mg Tea Tree Oil/L, based on fond number and dry weight, respectively. The 7-day E_rC_{50} value was estimated to be 10.3 mg Tea Tree Oil/L, based on fond number and dry weight. The NOEC was 4.63 mg Tea Tree Oil/L based on geometric mean measured concentrations.

Table 76: EC_{50}, LOEC and NOEC values of Tea Tree Oil technical for growth rate and yield

Frond number		**Dry weight**	
Growth rate inhibition [mg/L]			
NOEC	4.63	**NOEC**	1.91
LOEC	9.06	**LOEC**	4.63
EC_{r50} (95 % confidence limits)	10.3 (9.17 – 14.4)	**EC_{rdw50}** (95 % confidence limits)	10.3 (9.35 – 14.1)
Inhibition of yield [mg/L]			
NOEC	4.63	**NOEC**	1.91
LOEC	9.06	**LOEC**	4.63
EC_{y50} (95 % confidence limits)	10.0 (9.31 – 13.7)	**EC_{ydw50}** (95 % confidence limits)	10.6 (9.42 – 11.7)

Study 2: Anonymous (2017c), Tea Tree Oil (TTO): Alga, growth inhibition test with Pseudokirchneriella subcapitata, 72 h, OECD 201, GLP

Reliability statement: The study was performed in line with OECD 201 with no major deviations. All validity criteria were met and the test is considered acceptable (reliability score: 1).

The acute toxicity of Tea Tree Oil upon the growth of freshwater green algae *(Pseudokirchneriella subcapitata*) applied at 5 dose rates (2.56, 6.40, 16.0, 40.0 and 100 mg Tea Tree Oil/L) was observed over a period of 72 hours in a static test system. The study was carried out in closed bottles without headspace to avoid losses of the test item. Three replicate flasks at each test concentration and six control replicate flasks (Algal Growth Medium alone) were included in the test.

The 72-hour E_yC_{50} value for Tea Tree Oil was determined to be 1.76 mg Tea Tree Oil/L and the 72-hours E_rC_{50} value was estimated to be 2.17 mg Tea Tree Oil/L). The NOEC was 0.912 mg Tea Tree Oil/L based on geometric mean measured concentrations. Potential of recovery after exposure was observed at the geometric mean measured concentration of 3.15 mg Tea Tree Oil/L (algistatic effect) and an algicidal effect at the concentration levels of 7.45 and 19.8 mg Tea Tree Oil/L.

Table 77: Impact of Tea Tree Oil on mean cell density of *Pseudokirchneriella subcapitata*

Geometric mean measured Concentration of Tea Tree Oil [mg/L]	**Mean Cell Concentration [cells/mL] time [h]**			
	0	**24**	**48**	**72**
19.8	6305	n.a.	n.a	2407
7.45	6305	3996	n.a.	2913
3.15	6305	11166	7671	6203
1.26	6305	35797	203152	634166
0.912	6305	37246	209688	637905
Control	6305	40966	252889	716307

n.a. = data not applicable

Table 78: Evaluations of growth rate inhibition and yield inhibition after 72 h

Geometric mean measured Concentration of TTO [mg/L]	**Mean Growth Rate [day^{-1}]**	**Inhibition of Growth Rate [%]**	**Yield [cells/mL]**	**Inhibition of Yield [%]**
19.8	(+) -0.322	100	(+) -3898	100
7.45	(+) -0.260	100	(+) -3392	100
3.15	(+) - 0.006	99	(+) -102	100
1.26	(-) 1.54	3	(+) 627861	12
0.912	(-) 1.54	3	(-) 631600	11
Control	1.58	-	710002	-

(-) = statistically non-significant differences compared to the control values.
(+) = statistically significant differences compared to the control values.

11.5.4 Acute (short-term) toxicity to other aquatic organisms

No further relevant aquatic effects data are available.

11.6 Long-term aquatic hazard

Table 79: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results [mg as/L]	Remarks	Relia-bility score	Reference
Early life stage test with fish OECD 210 GLP Flow-through, ELS	*Pimephales promelas*	Tea Tree Oil Purity: 10.3% α-Terpinene, 20.9% γ-Terpinene, 1.36% 1,8-Cineole, 1.53% p-Cymene and 42.36% Terpinen-4-ol. (in compliance with ISO specification)	NOEC = 0.244	measured	1	Anonymous (2017e)
Reproductive and developmental toxicity to *Daphnia* OECD 211 GLP Semi-static, 21d	*Daphnia magna*	Tea Tree Oil Purity: 10.3% α-Terpinene, 20.9% γ-Terpinene, 1.36% 1,8-Cineole, 1.53% p-Cymene and 42.36% Terpinen-4-ol. (in compliance with ISO specification)	NOEC = 0.303 EC_{10} = 0.411	measured	1	Anonymous (2017f)
Development and emergence in *Chironomus* OECD 219 GLP Water-sediment, 28d	*Chironomus riparius*	Tea Tree Oil Purity: 10.3% α-Terpinene, 20.9% γ-Terpinene, 1.36% 1,8-Cineole, 1.53% p-Cymene and 42.36% Terpinen-4-ol. (in compliance with ISO specification)	NOEC = 4.36 NOEC = 13.32 mg as/kg sediment EC_{50} = 28.3 EC_{50} = 86.47 mg as/kg sediment	measured	1	Anonymous (2017g)
Algae growth inhibition test OECD 201, GLP Static, 72 h	*Pseudo-kirchneriella subcapitata*	Tea Tree Oil Purity: 10.3% α-Terpinene, 20.9% γ-Terpinene, 1.36% 1,8-Cineole, 1.53% p-Cymene and 42.36% Terpinen-4-ol. (in compliance with ISO specification)	NOEC = 0.912	measured	1	Anonymous (2017c)
Aquatic plant toxicity test OECD 221, GLP	*Lemna gibba*	Tea Tree Oil Purity: 10.3% α-Terpinene, 20.9% γ-Terpinene, 1.36% 1,8-Cineole, 1.53% p-Cymene and 42.36% Terpinen-4-ol (in compliance with ISO specification)	NOEC = 1.91	measured	1	Anonymous (2017d)

11.6.1 Chronic toxicity to fish

One chronic toxicity study on Tea Tree Oil (early life stage test with fish) to *Pimephales promelas* leads to a NOEC of 0.244 mg as/L.

Study 1: Anonymous (2017e), Tea Tree Oil (TTO): Early-Life Stage Toxicity Test with Fathead Minnow (Pimephales promelas) under Flow-Through Conditions, OECD 210, GLP

Reliability statement: The study was performed according to OECD 210 with no major deviations. All validity criteria were met and the study is considered acceptable (reliability score: 1).

The effects of the test item Tea Tree Oil (TTO) to the early-life stage of fish (*Pimephales promelas* / Fathead minnow) were determined according to OECD Guideline 210.

A test was conducted under flow-through conditions with the nominal test item concentrations of 0.640, 1.60, 4.00, 10.0 and 25.0 mg/L, corresponding to arithmetic mean measured concentrations of 0.244, 0.373, 0.885, 1.40 and 3.15 mg/L. Due to the low solubility of the test item in water, methanol was used as solvent with a loading of 0.10 mL per L dilution water.

The test was started by placing fertilized eggs into the test vessels and lasted 34 days (28 days post-hatch). 80 eggs of *Pimephales promelas* were exposed to each test concentration, the solvent control and the control (4 replicates with 20 eggs each).

The water quality parameters pH-value, oxygen concentration, temperature and total hardness were within the acceptable limits.

On study day 6, 85% of the control and 86% of the solvent control larvae had hatched. Therefore, study day 6 was defined as post hatch day 0 (= PHD 0).

Different toxicological endpoints were determined: hatch, time to hatch, fry growth (expressed as length and fresh weight), morphological and behavioural effects and post-hatch survival.

Specific analysis of various concentrations of TTO in the test media and the control groups was carried out via SPME-GC-MS.

The test media were sampled and analysed prior to exposure on day -1 and during the exposure on study days 0, 6, 14, 21, 23, 28 and 33. The measured concentrations of the test media during the exposure were in the range of 5% to 49% of the nominal values.

All effect values are given based on arithmetic mean measured concentrations of the test item Tea Tree Oil.

The results of the parameters hatch, time to hatch, fry growth (expressed as weight and length) and post-hatch survival were checked for statistically significant differences. The effect values NOEC and LOEC were determined based on the statistical results. The results are presented in the table below:

Table 80: Hatch, fry growth, fry survival: NOEC, LOEC and LC_{50} Based on arithmetic mean measured test item concentrations [mg/L]

Parameter	NOEC	LOEC	LC_{50} (95% C.I.)
Hatch	0.373	0.885	
Fry Growth expressed as: Length Weight	 ≥ 3.15 ≥ 3.15	 > 3.15 > 3.15	
Post hatch survival	≥ 3.15	> 3.15	
Cumulative survival	0.244	0.373	0.818 (0.500 - 0.884)

11.6.2 Chronic toxicity to aquatic invertebrates

One chronic toxicity study on Tea Tree Oil to *Daphnia magna* is available and leads to a NOEC of 0.303 mg as/L and a corresponding EC_{10} of 0.411 mg as/L.

Study 1: Anonymous (2017f), Tea Tree Oil (TTO): Daphnia magna reproduction test, semi-static, 21 days, in a closed system without headspace, OECD 211, GLP

Reliability statement: The study was performed in line with OECD 211 with no major deviations. All validity criteria were met and the test is considered acceptable (reliability score: 1).

A *Daphnia magna* reproduction test (semi-static, 21 d) with Tea Tree Oil (TTO) was conducted according to OECD 211 (2012).

Ten daphnids (2 to 24 hours old) held individually, were used per concentration level and control.

The study was carried out in a closed system without headspace (sealed glass flasks filled up to the top with the test solutions) under semi-static conditions with a daily renewal of the test solutions. The aim of the test was to assess the effects on the reproduction capacity and other sub-lethal effects.

Five concentration levels of the test item TTO were tested, prepared in a geometric series with a separation factor of 2.5: 0.640, 1.60, 4.00, 10.0, 25.0 mg/L.

The concentrations of TTO were analytically verified via GC-MS in fresh media on days 0, 7, 14 (0 hours) and in old media on days 1, 8, 15 (24 hours) in all concentration levels with surviving parental daphnids and the control.

The measured concentrations of the test item in the fresh media (0 hours) were in the range of 17 to 25% of the nominal concentrations. At the end of the respective exposure intervals (24 hours), the measured concentrations in the old media were in the range of 13 to 34% of the nominal values.

The geometric mean measured concentrations of the test item were: 0.132 - 0.303 - 0.747 – 2.02 - 5.75 mg/L.

The effect-concentrations ($EC_{10\ /\ 50}$, $LC_{20\ /50\ /\ 100}$, LOEC and NOEC) were based on the geometric mean measured concentrations of the test item.

The test item induced statistically significant adult mortality of 40% in the concentration level 0.747 mg/L and 100% in the concentration levels of 2.02 and 5.75 mg/L. In the two lowest concentration levels of 0.132 and 0.303 mg/L and in the control all parental daphnids survived until the end of the test (21 days).

A statistically significant reduction of the reproductive output in comparison to the reproductive output in the control was determined at the three highest concentration levels of 0.747 to 5.75 mg/L. At the two lowest concentration levels of 0.132 and 0.303 mg/L, the reproductive output was comparable to the reproductive output of the control.

A summary of all endpoints based on the geometric mean measured concentrations of the test item Tea Tree Oil (TTO) is given in the following table.

Table 81: Endpoints for Reproduction and Mortality (based on the geometric mean measured concentrations of the test item)

Effect values	Tea Tree Oil (TTO)
$EC_{10\ Reproduction}$ (with 95% confidence limits)	**0.411** (0.0838 2.02)
$EC_{50\ Reproduction}$ (with 95% confidence limits)	0.531 (0.0998 - 2.95)
$LOEC_{\ Reproduction}$	0.747

Effect values	Tea Tree Oil (TTO)
NOEC Reproduction	**0.303**
LC_{20} Adult mortality after 21 days (with 95% confidence limits)	0.677 (Not applicable)
LC_{50} Adult mortality after 21 days (with 95% confidence limits)	0.779 (Not applicable)
LC_{100} Adult mortality after 21 days	2.02
LOEC Adult mortality after 21 days	0.747
NOEC Adult mortality after 21 days	0.303

11.6.3 Chronic toxicity to algae or other aquatic plants

Please refer to previous point 11.5.3 where the toxicity tests with the substance on algae are included.

11.6.4 Chronic toxicity to other aquatic organisms

One chronic toxicity study on the effects of Tea Tree Oil to *Chironomus riparius* is available and lead to a NOEC of 4.36 mg as/L and a corresponding EC_{50} of 28.3 mg as/L.

Study 1: Anonymous (2017g), Tea Tree Oil (TTO): Sediment-water chironomid toxicity test using spiked water

Reliability statement: The study was performed in line with OECD 219 with no major deviations. All validity criteria were fulfilled and the test is considered acceptable (reliability score: 1).

The effects of the test item Tea Tree Oil (TTO) on the development of the common non-biting midge *Chironomus riparius* in a water-sediment system were determined. The study was carried out according to the principles of OECD Guideline 219.

A dose response test was conducted by spiking the water layer. Five test item concentrations (nominal concentrations in the aqueous layer) of 10.2, 25.6, 64, 160, 400 mg/L (factor of 2.5) corresponding to initial measured concentrations of 1.91, 4.36, 9.56, 27.8, 73.4 mg/L were tested. 100 first instar larvae were exposed to each test concentration and to the control (5 replicates with 20 larvae each, 4 for the biological part and one for the analytical part on day 7).

Water quality parameters such as temperature, pH-value and O_2-content were determined regularly throughout the study. Also, ammonium and total hardness were analysed at the day of application (day 0) and at test end (day 28) from the control and the highest test item group.

The concentrations of the test item were analytically verified via GC-MS on days 0, 7 and 28. The test item was analysed in the sediment layer after extraction and liquid injection. Analysis of pore water and overlying water phase were carried out via SPME injection.

In the control, emergence of the midges started 13 days after larvae insertion and was finished at day 20 after larvae insertion with an emergence ratio of 94%. In the test item concentrations, emergence of the midges started 13 days after larvae insertion and was finished at day 23 after larvae insertion and the emergence ratio ranged from 0 to 95%.

Tea Tree Oil did affect the emergence rate and development rate of *Chironomus riparius* at the test item concentrations of 27.8 mg/L and higher compared to the control. At 73.4 mg/L no mean emergence rate and development rate was determinable.

The results indicate a NOEC for the emergence rate at 9.56 mg/L and the development rate at 4.36 mg/L. The EC_{50} for mortality is determined 28.3 mg/L. A summary of all endpoints based on the measured concentrations of the test item TTO is given in the following table.

Table 82: Effect levels after 28 d (based on initial measured concentrations of the test item)

Effect values [mg/L]	**Tea Tree Oil (TTO)**
EC_{50} (Emergence)	28.3
LOEC (Emergence Rate) Lowest tested concentration with an observed effect on the emergence rate after 28 d	27.8
NOEC (Emergence Rate) Highest tested concentration without any observed effect on the emergence rate after 28 d	9.56
LOEC (Development Rate) Lowest tested concentration with an observed effect on the development rate after 28 d	9.56
NOEC (Development Rate) Highest tested concentration without any observed effect on the development rate after 28 d	4.36

Although in the current study the "No Observed Effect Concentration' (NOEC) for the emergence rate (9.56 mg/L) and the development rate (4.36 mg/L) have been only expressed in mg/L, based on the water overlying the sediment (i.e. 550 ml per test vessel), the corresponding NOEC values, based on the sediment, could be calculated by taking into account the test sediment amount of 180 g dry weight per test vessel. Therefore, the NOEC for the emergence rate and the development rate, based on the sediment, could be calculated as 29.21 mg/kg sediment dry weight and 13.32 mg/kg sediment dry weight, respectively.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Aquatic acute toxicity data on Tea Tree Oil are available for fish, invertebrates, algae and higher aquatic plants. Daphnids are the most acutely sensitive trophic group with EC_{50} values ≤ 1.0 mg/L. The lowest value is 0.591 mg/L for *Daphnia magna*. On this basis, Tea Tree Oil meets the criteria from the CLP regulation (Annex I, section 4.1, table 4.1.0) for classification in Category Acute 1.

As the lowest acute toxicity endpoint is within Regulation (EC) 1272/2008 criteria $0.1 < L(E)C_{50} \leq 1$ mg/L (see Table 4.1.3) the corresponding Acute M-factor is 1.

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Degradation

As summarised in section 11.1.1. Tea Tree Oil is considered rapidly degradable according to CLP criteria.

Bioaccumulation

Estimated BCF is < 500 for all monoterpene components, which account on average for > 95% of Tea Tree Oil. For the sesquiterpenes, BCF > 500 has been estimated, however, for the majority of these below 600, i.e. close to the trigger of 500. The sesquiterpene content of Tea tree oil is traces to max. 3.5% (individually), and cumulatively usually < 5%. Cumulative content of components with BCF > 600 (Cadinene, Aromadendrene and Ledene, BCF range 5000-7000) usually is below 2%.

Overall, Tea Tree Oil is considered to be not bioaccumulative. On this basis, the substance does not meet CLP criteria as a bioaccumulative substance.

Chronic toxicity

As discussed in sections 11.6.1, 11.6.2, 11.6.3 and 11.6.4 there are reliable chronic toxicity endpoints for fish, aquatic invertebrates, algae, aquatic plants and sediment dweller.

The lowest chronic endpoint is for fish, i.e. the fish early life stage NOEC endpoint of 0.244 mg as/L. As this endpoint is within classification criteria > 0.1 to <1 mg/L (Table 4.1.0) and as Tea Tree Oil is considered rapidly degradable (section 11.1.1.), the corresponding chronic classification is Chronic Category 3. No relevant Chronic M-factor is appointed to this category.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Based on toxicity data, Tea Tree Oil should be classified as Category Acute 1 (Acute M Factor 1), Category Chronic 3 (Chronic M Factor: none) and labelled with hazard statement H410 Very toxic to aquatic life.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Not considered in this assessment.

12.1.1 Short summary and overall relevance of the provided information on ozone layer hazard

12.1.2 Comparison with the CLP criteria

12.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

Not considered in this assessment.

13 ADDITIONAL LABELLING

No additional labelling proposed.

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Anonymous 2017c	2017	Algae, growth inhibition test with *Pseudokirchneriella subcapitata*, 72 hours Noack-Laboratorien, Germany Report No.: SPO16943 GLP, not published

14 ANNEXES

Four annexes are provided with this report, which are compiled from the draft registration report of the AIR 4 substance renewal of Tea Tree Oil that was submitted to the RMS Poland in February 2018.

Only parts that are relevant for the CLH report are included.

The annexes cover the physical-chemical properties, the toxicological, environmental and ecotoxicological parts of the draft registration report.

REACH registration dossier – CSR 2020